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(54) **Xenobiotic delivery vehicles, method of forming them and method of using them**

(57) A delivery vehicle, suitable for delivering and releasing a xeno- biotic to a mammalian host, to be- neficially alter the pharmacodynam- ics (e.g. plasma kinetics, chemo- therapeutic effectiveness, toxicity, oral absorption, tissue distribution, metabolism and the like) of the xe- nobiotic, is in the form of microres- ervoirs formed of a phospholipid constituent and a phospholipid-im- miscible constituent. Xenobiotic binding agents and release agents may be added.
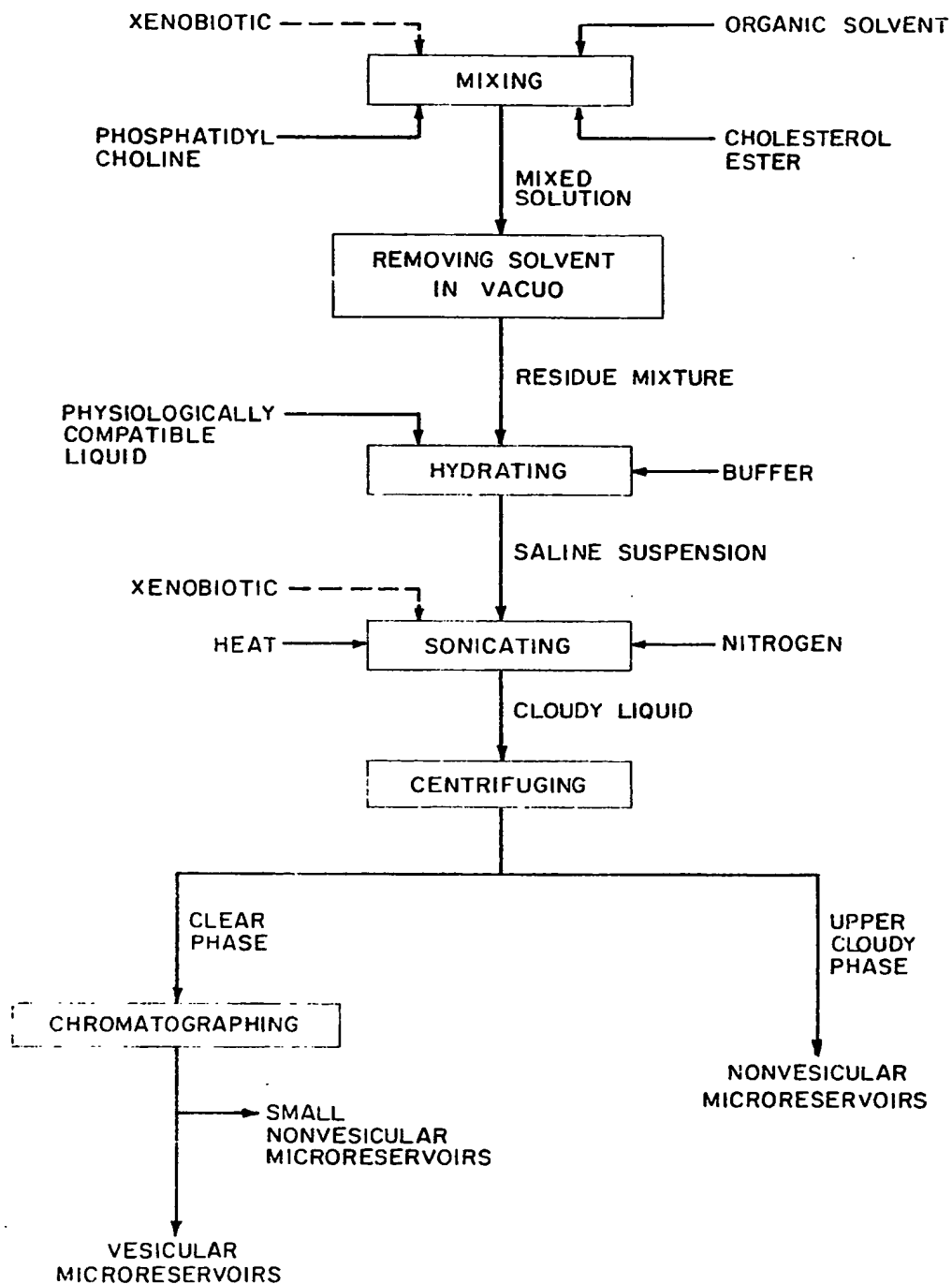
XENOBIOTIC — — — — ┐                    ┌— ORGANIC SOLVENT

┌─────────────────┐
│     MIXING      │
└─────────────────┘

PHOSPHATIDYL─────┘                      └── CHOLESTEROL
CHOLINE                                      ESTER

MIXED
SOLUTION

┌─────────────────┐
│ REMOVING SOLVENT │
│    IN  VACUO     │
└─────────────────┘

RESIDUE MIXTURE

PHYSIOLOGICALLY ──────┐
COMPATIBLE
LIQUID

┌─────────────────┐
│    HYDRATING    │◄────── BUFFER
└─────────────────┘

SALINE SUSPENSION

XENOBIOTIC — — — — ┐

┌─────────────────┐
HEAT ─────────►│   SONICATING    │◄────── NITROGEN
└─────────────────┘

CLOUDY LIQUID

┌─────────────────┐
│   CENTRIFUGING  │
└─────────────────┘

CLEAR                                    UPPER
PHASE                                    CLOUDY
                                         PHASE

┌─────────────────┐
│ CHROMATOGRAPHING │                     NONVESICULAR
└─────────────────┘                     MICRORESERVOIRS

──► SMALL
     NONVESICULAR
     MICRORESERVOIRS

VESICULAR
MICRORESERVOIRS

Fig. I

AQUEOUS ENVIRONMENT



O—< PHOSPHATIDYL CHOLINE
AND CHOLESTEROL

**Fig. 5**

XENOBIOTIC — — — — — — — —
PHOSPHOLIPID ——————
CHOLESTERYL ——→ MIXING
ESTER
SOLVENT ——————  SOLUTION

PHYSIOLOGICALLY
COMPATIBLE
LIQUID              INJECTING
(CONTAINING
XENOBIOTIC )      CLOUDY LIQUID

CENTRIFUGING

**Fig. 2**

AQUEOUS ENVIRONMENT



O—< PHOSPHATIDYL CHOLINE
O~ CHOLESTEROL ESTER

**Fig.3**

AQUEOUS ENVIRONMENT



O—< PHOSPHATIDYL CHOLINE
O~ CHOLESTEROL ESTER

**Fig. 4**
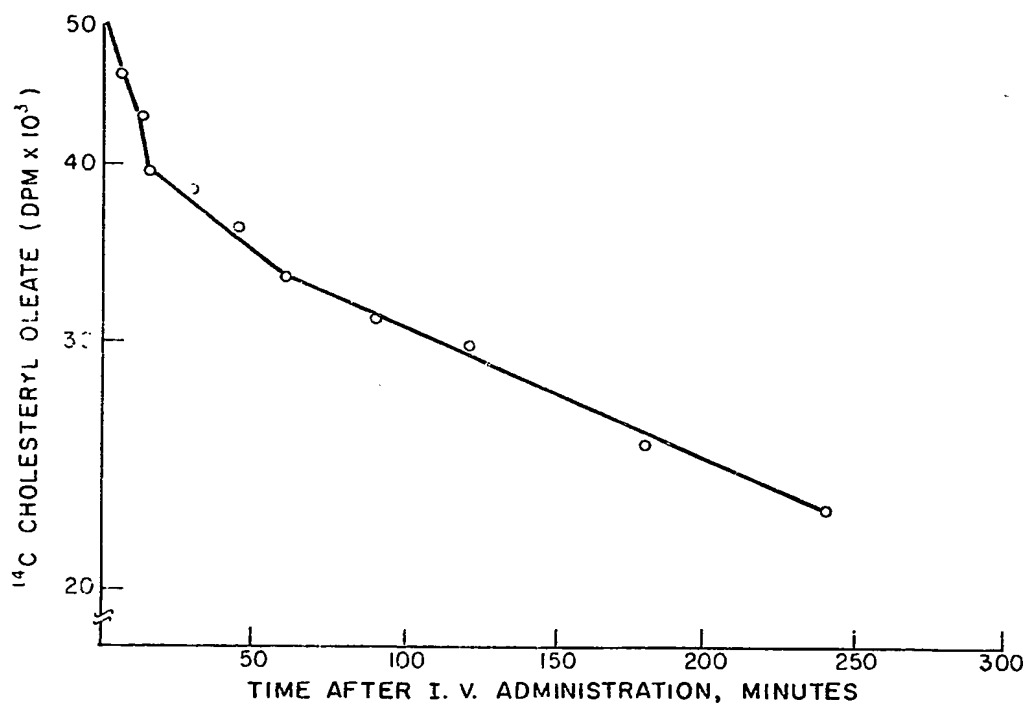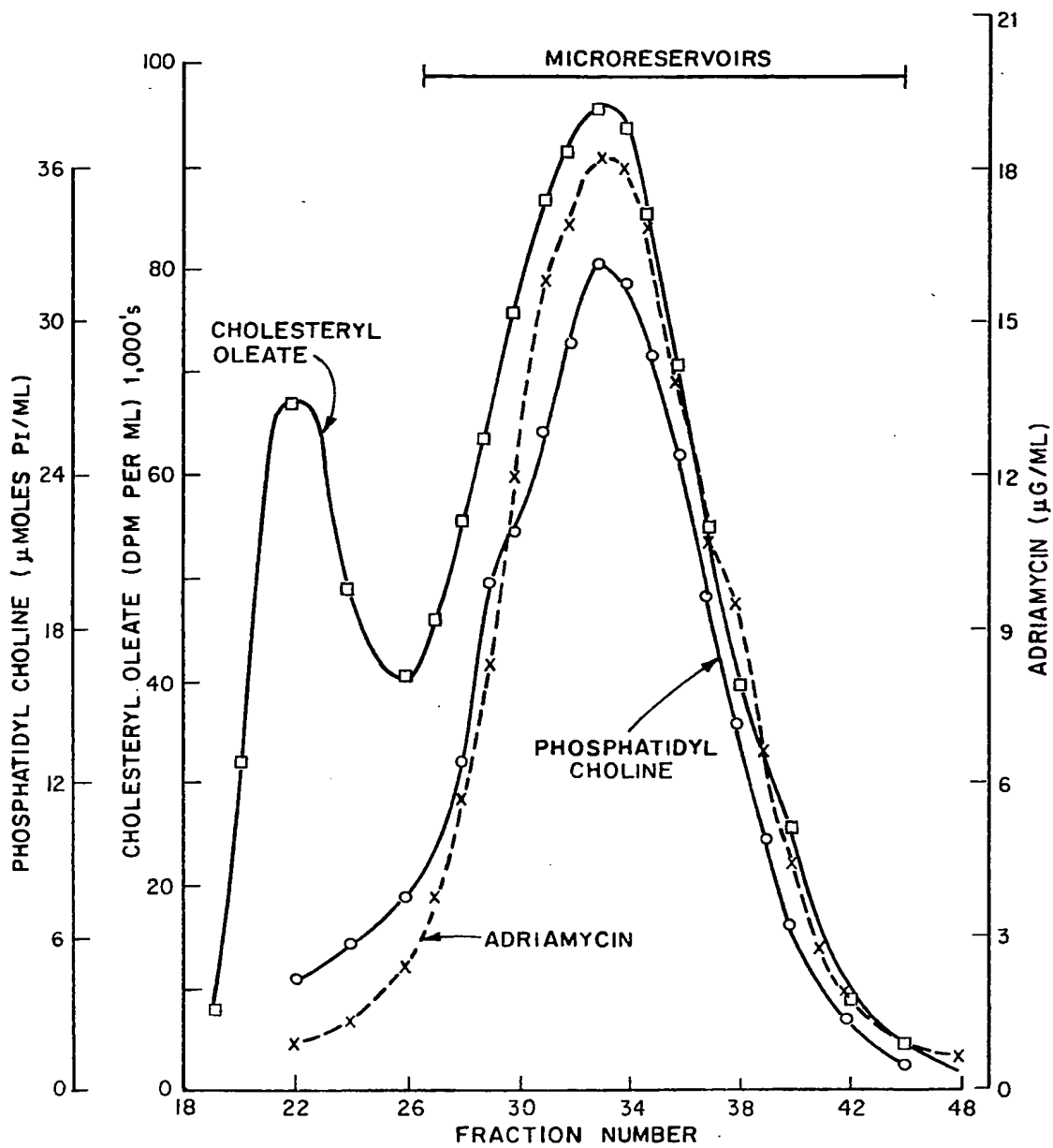
IN VITRO STABILITY OF MICRORESERVOIRS
AND PRIOR ART LIPOSOMES

Fig. 6



IN VIVO STABILITY OF MICRORESERVOIRS

Fig. 7
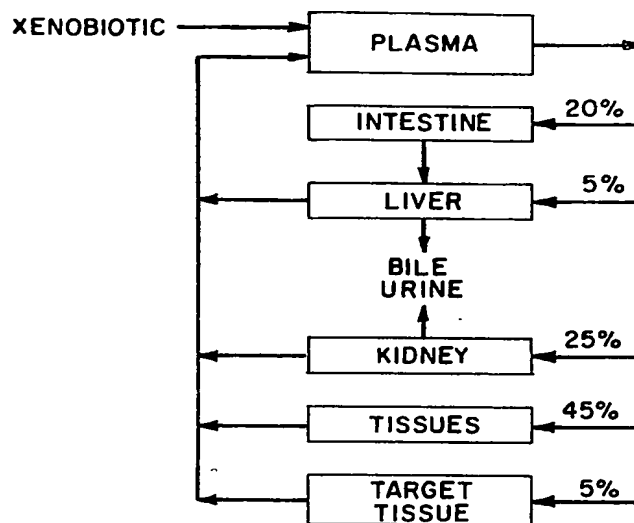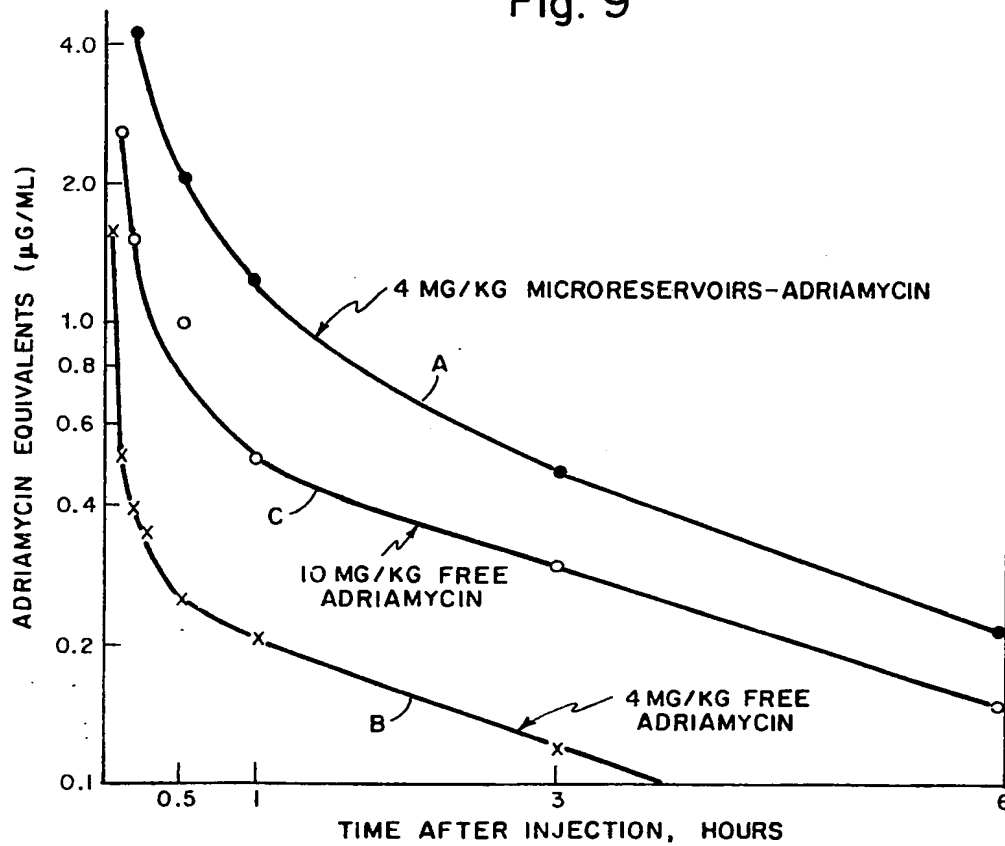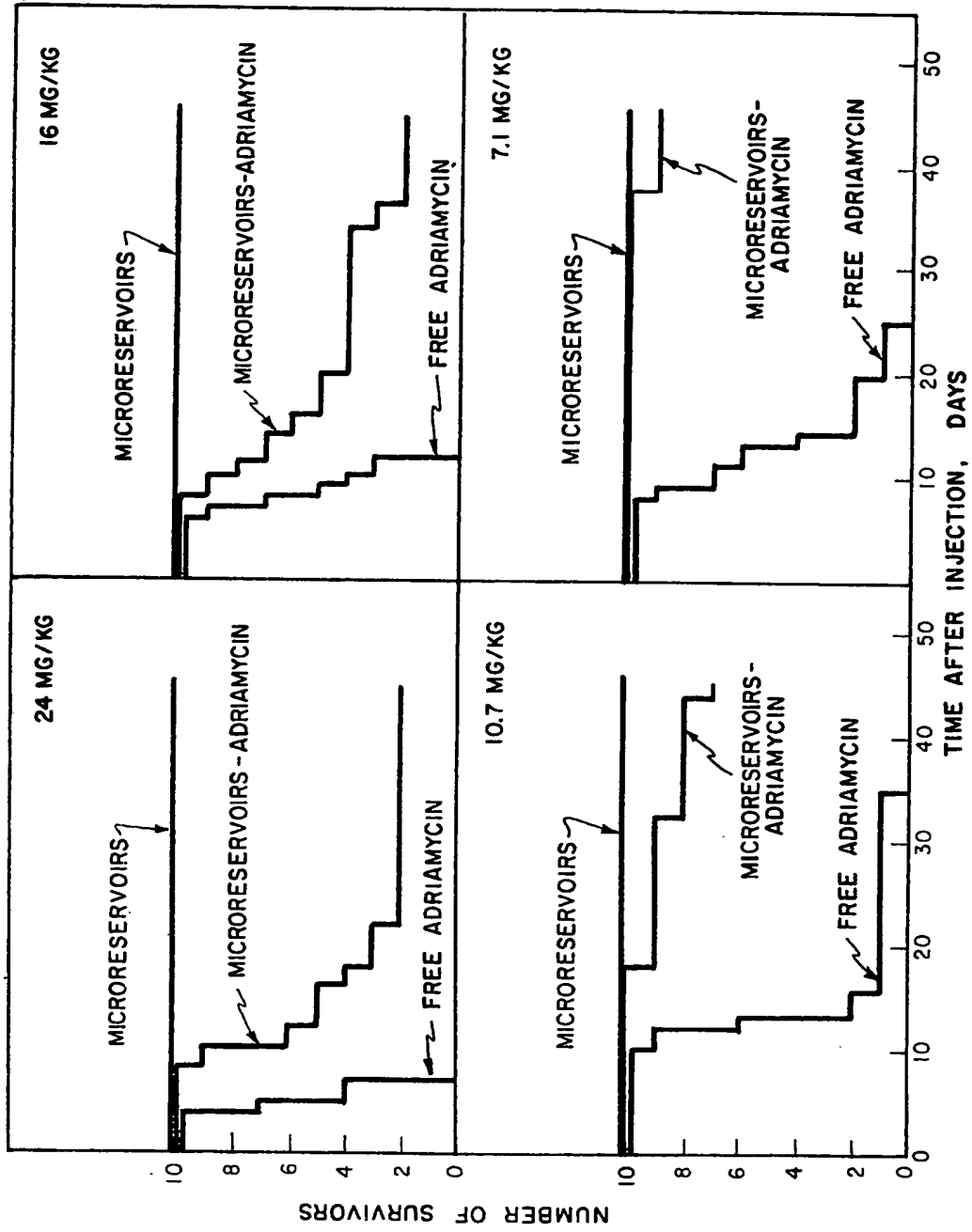
ELUTION PROFILE OF MICRORESERVOIRS-ADRIAMYCIN

Fig. 8

XENOBIOTIC ⟶ | PLASMA |

| INTESTINE | ← 20%

| LIVER | ← 5%

BILE
URINE

| KIDNEY | ← 25%

| TISSUES | ← 45%

| TARGET
TISSUE | ← 5%

## Fig. 9



4 MG/KG MICRORESERVOIRS-ADRIAMYCIN

A

C

IO MG/KG FREE
ADRIAMYCIN

4 MG/KG FREE
ADRIAMYCIN

B

ADRIAMYCIN EQUIVALENTS (µG/ML)

TIME AFTER INJECTION, HOURS

PLASMA KINETICS OF MICRORESERVOIRS-ADRIAMYCIN
AND FREE ADRIAMYCIN

## Fig. IO

TOXICITY OF MICRORESEVOIRS-ADRIAMYCIN AND FREE ADRIAMYCIN

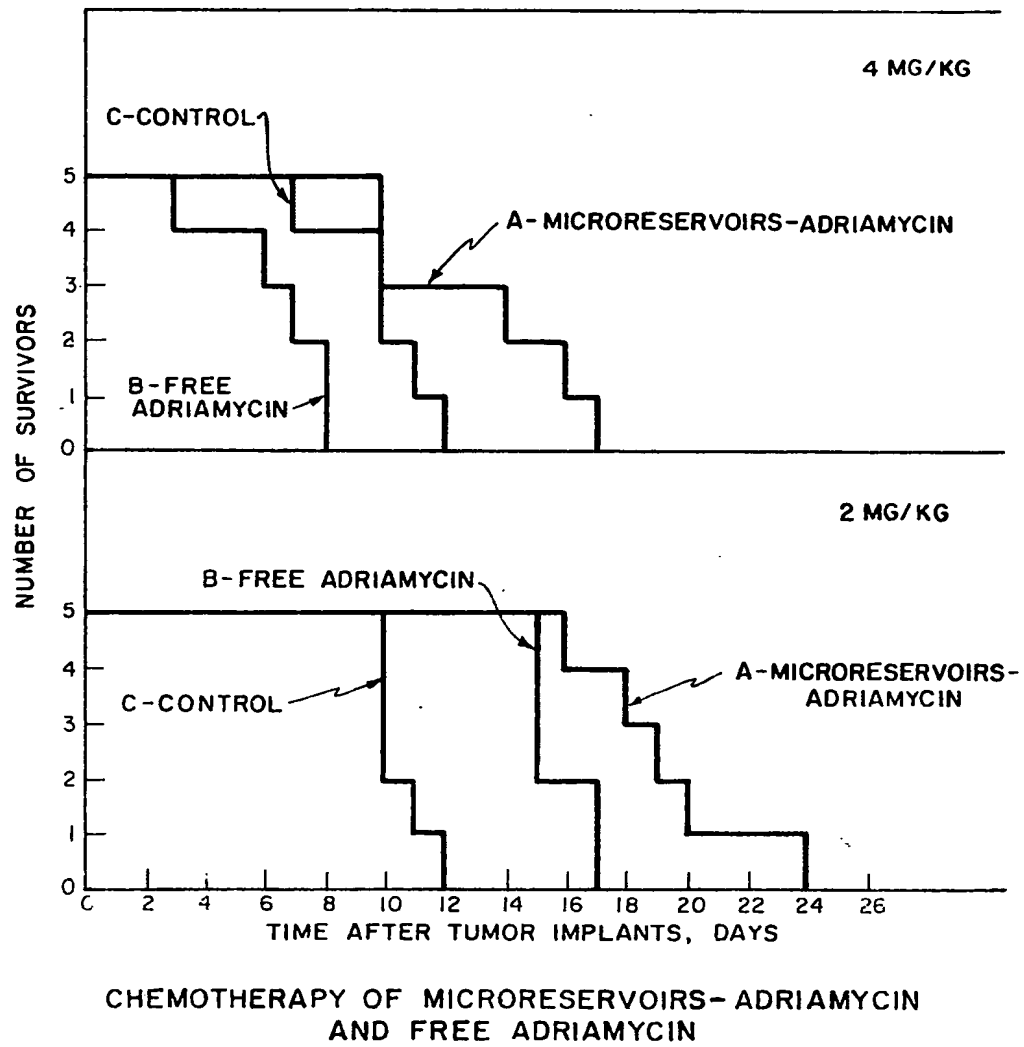Fig. 11

CHEMOTHERAPY OF MICRORESERVOIRS- ADRIAMYCIN
AND FREE ADRIAMYCIN
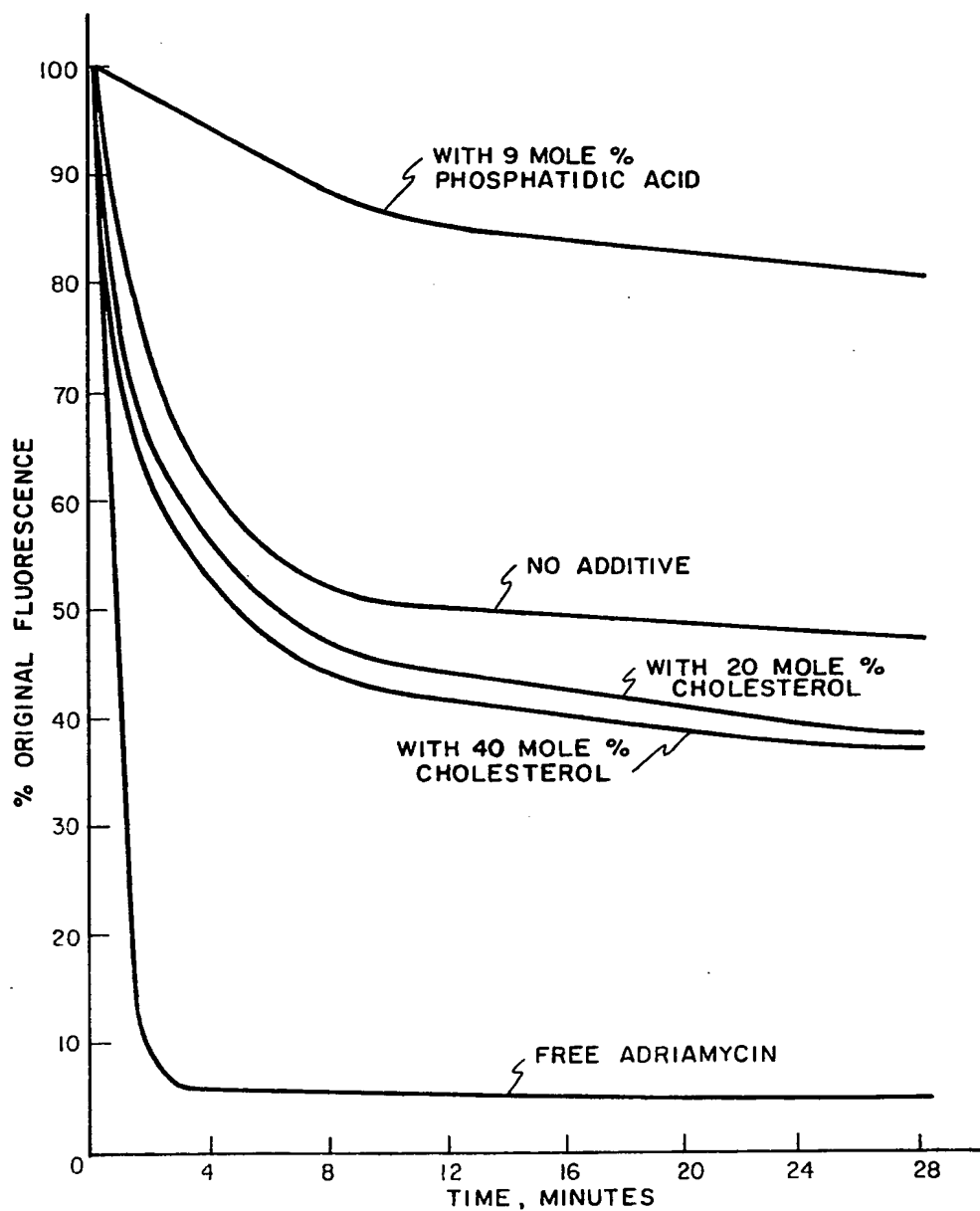
Fig. 12

EFFLUX RATE OF ADRIAMYCIN FROM MICRORESERVOIRS FORMED
OF PHOSPHATIDYL CHOLINE AND GLYCEROL TRIOLEATE
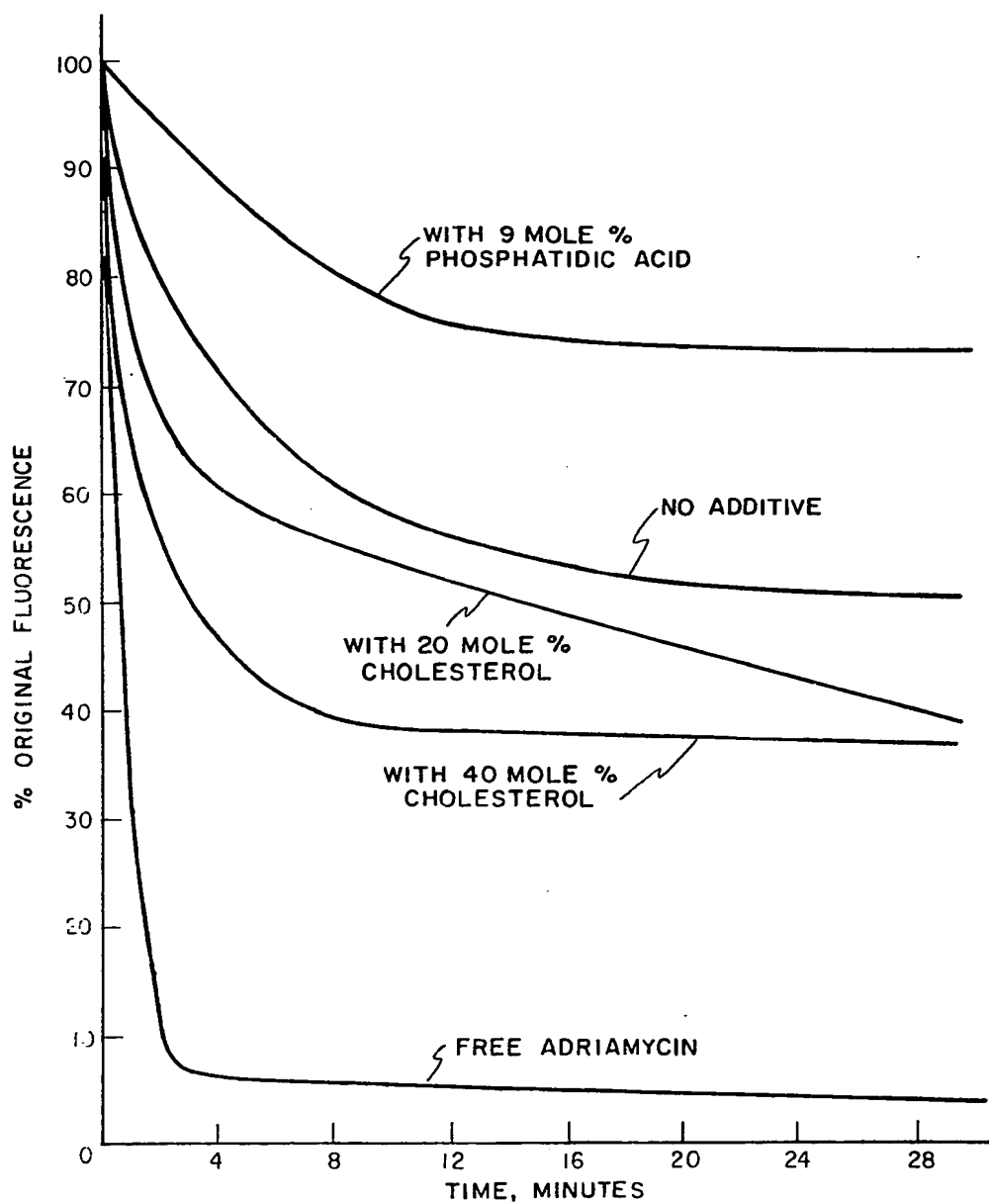
Fig. 13

EFFLUX RATE OF ADRIAMYCIN FROM MICRORESERVOIRS FORMED
OF PHOSPHATIDYL CHOLINE AND CHOLESTERYL OLEATE

Fig. 14

ELUTION PROFILE OF MICRORESERVOIRS-AD 32
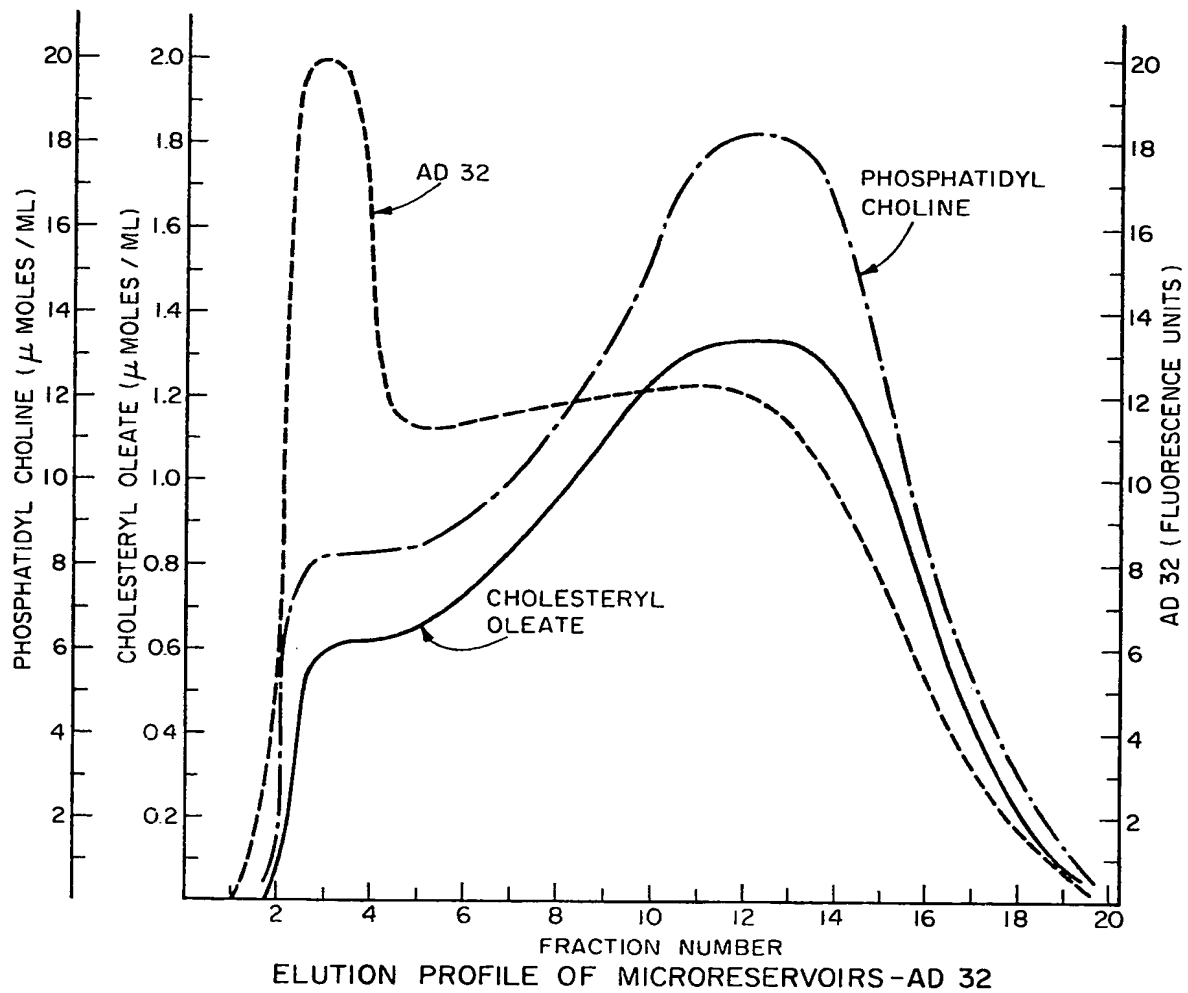
Fig. 15
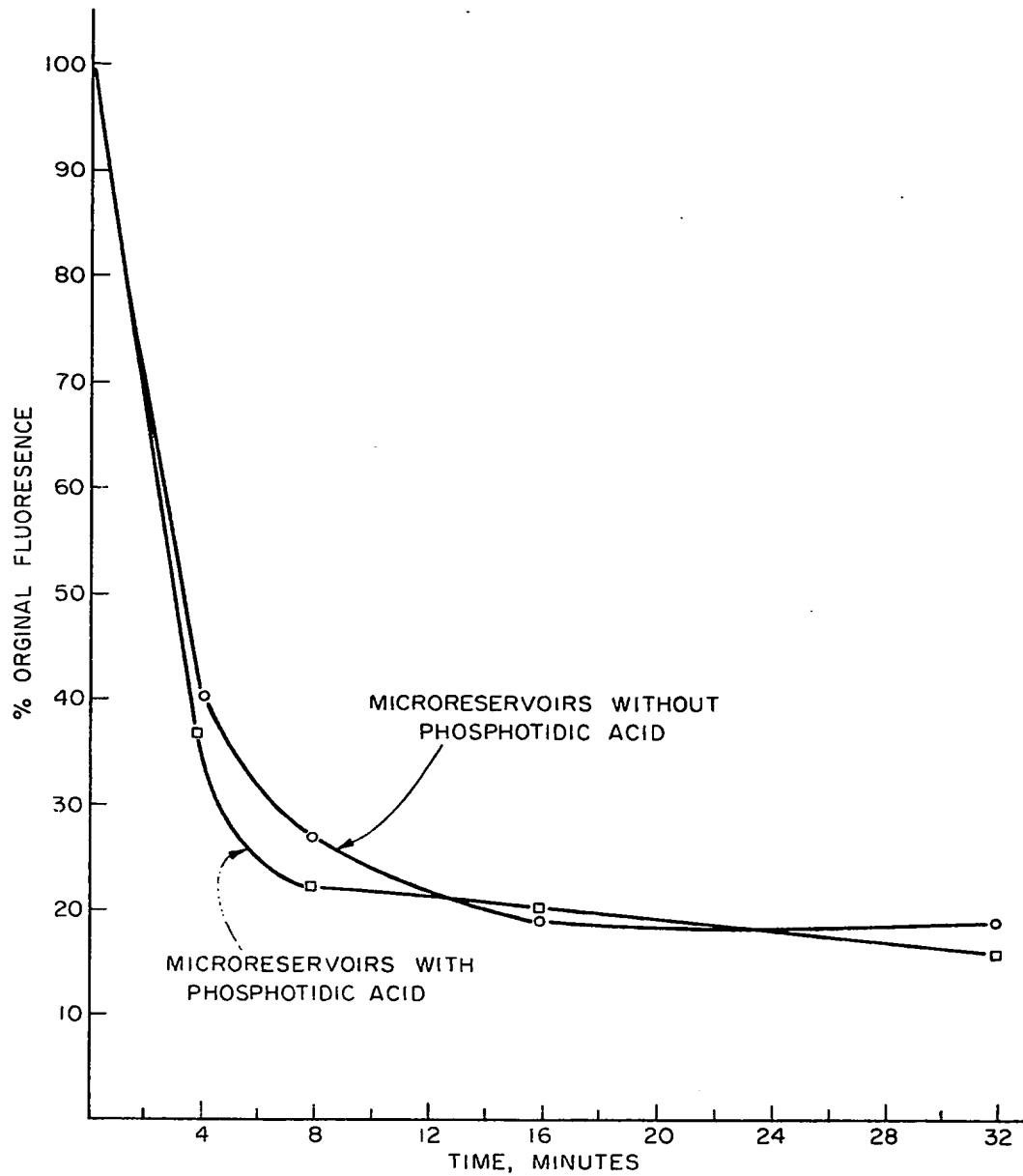
EFFLUX RATE OF AD 32 FROM MICRORESERVOIRS
FORMED WITH AND WITHOUT PHOSPHOTIDIC ACID

Fig.16

PLASMA KINETICS OF MICRORESERVOIRS-AD 32
AND FREE AD 32-DOSE OF 15 MG/KG
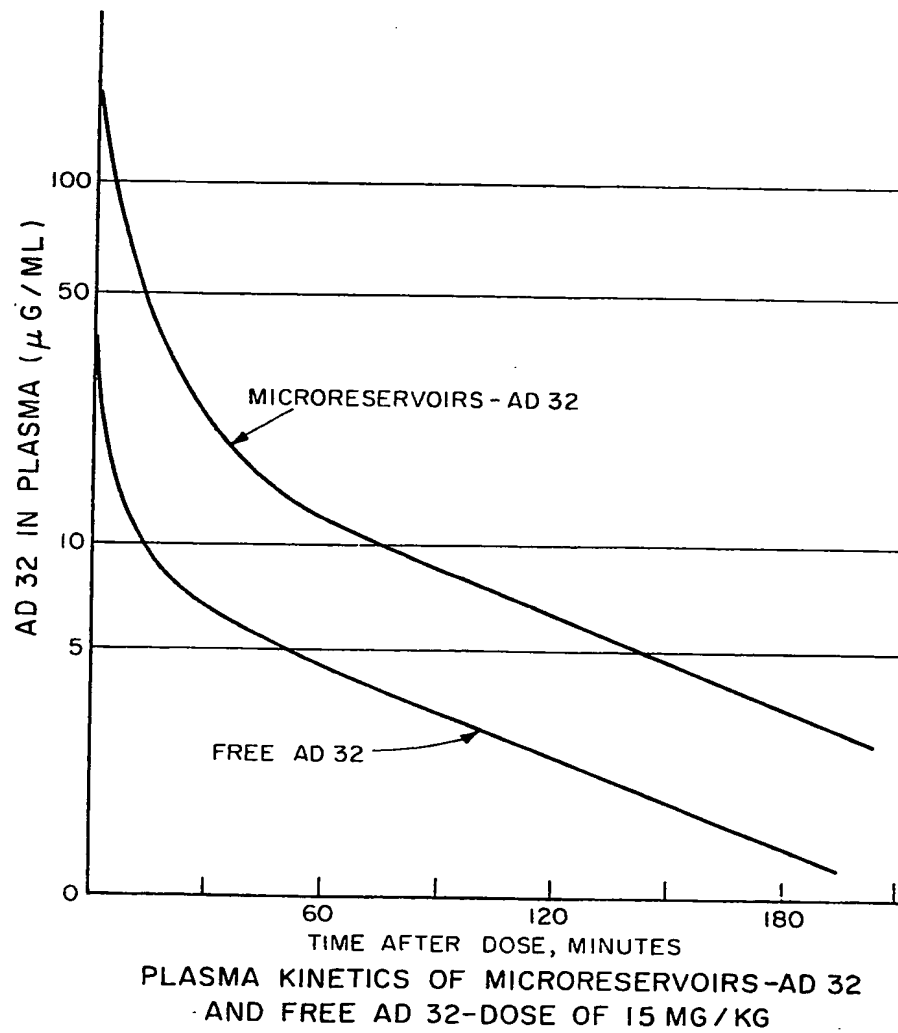
Fig. 17
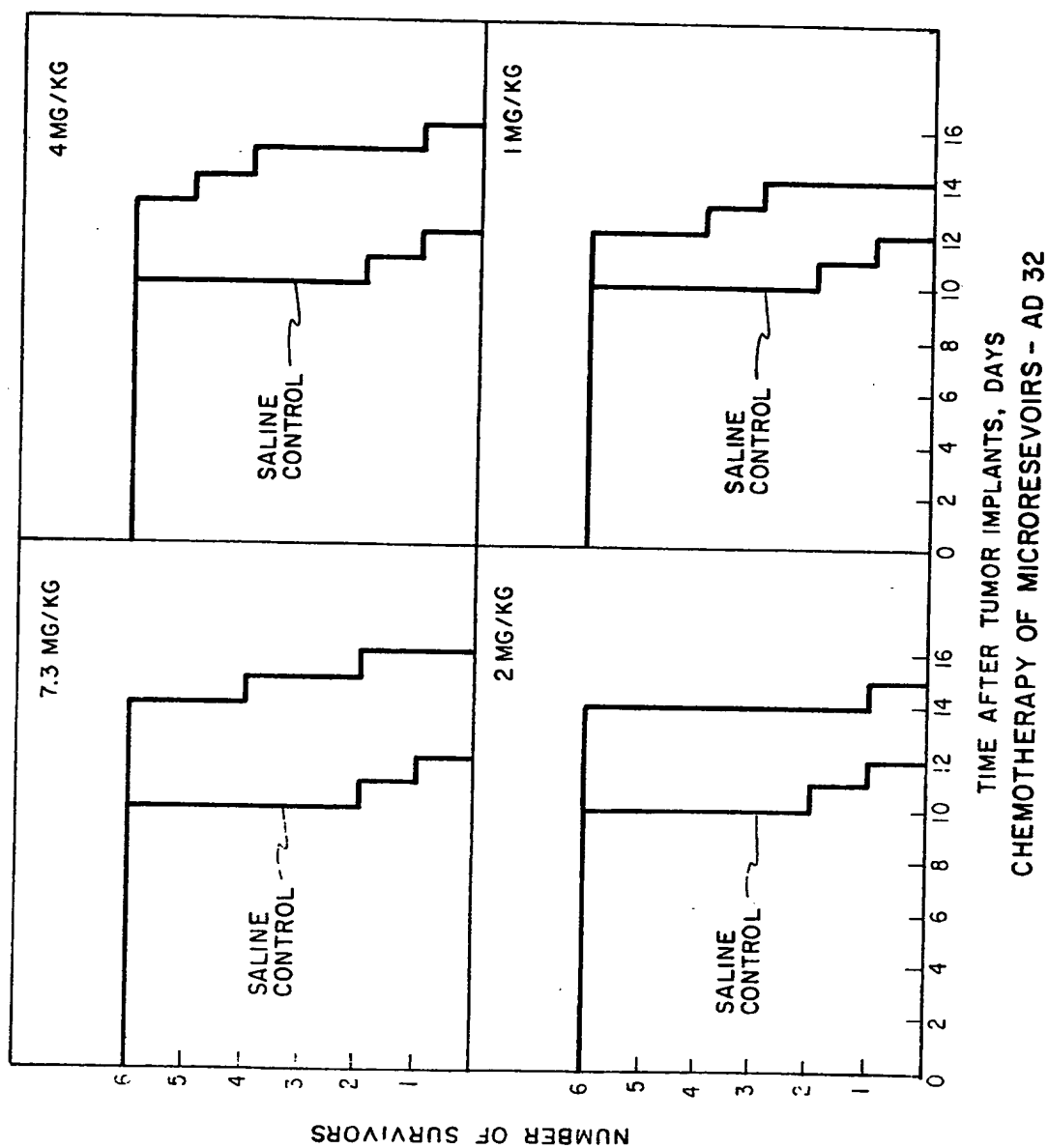
Fig. 18
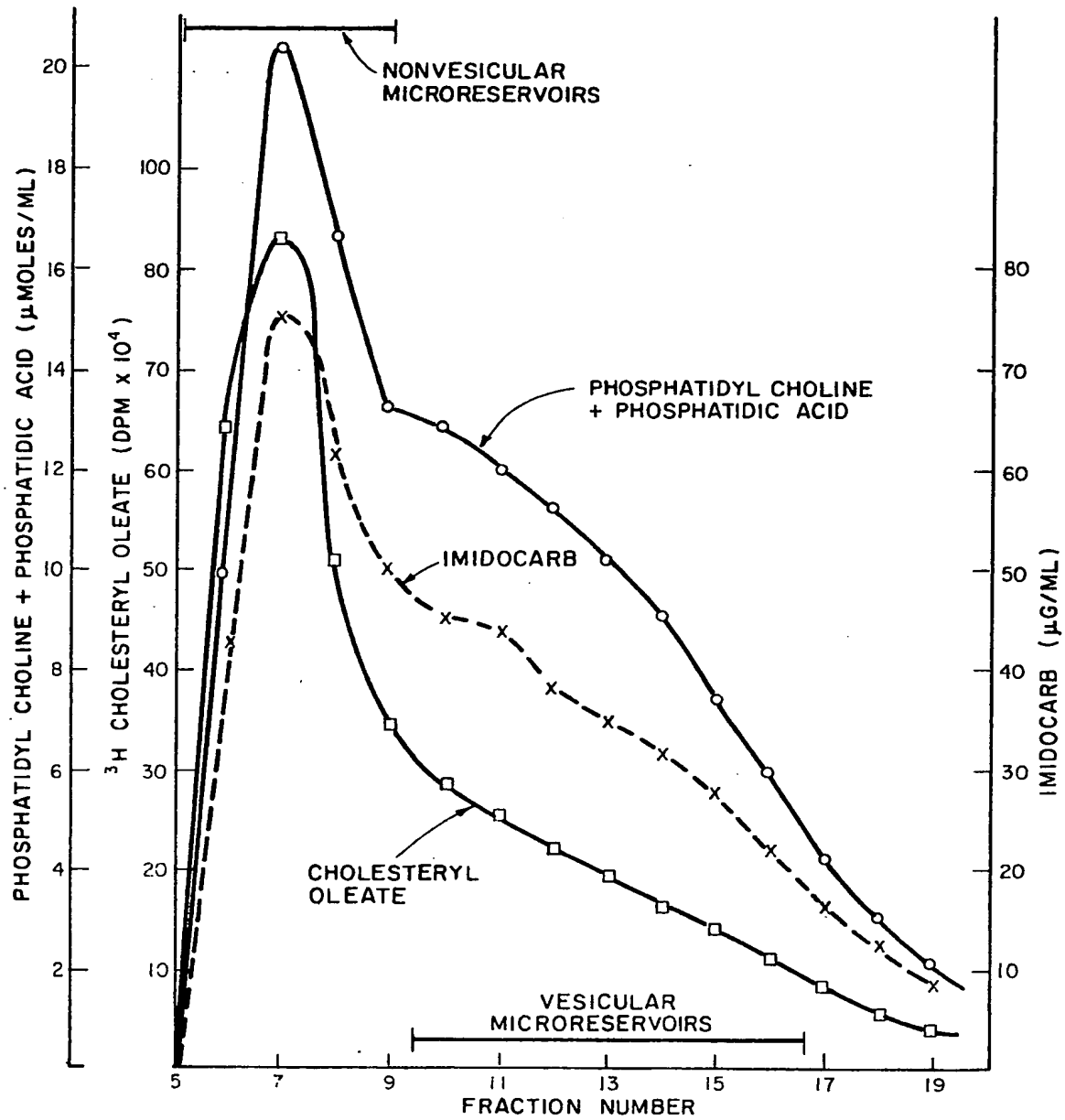
ELUTION PROFILE OF MIRCORESERVOIRS – IMIDOCARB

Fig. 19
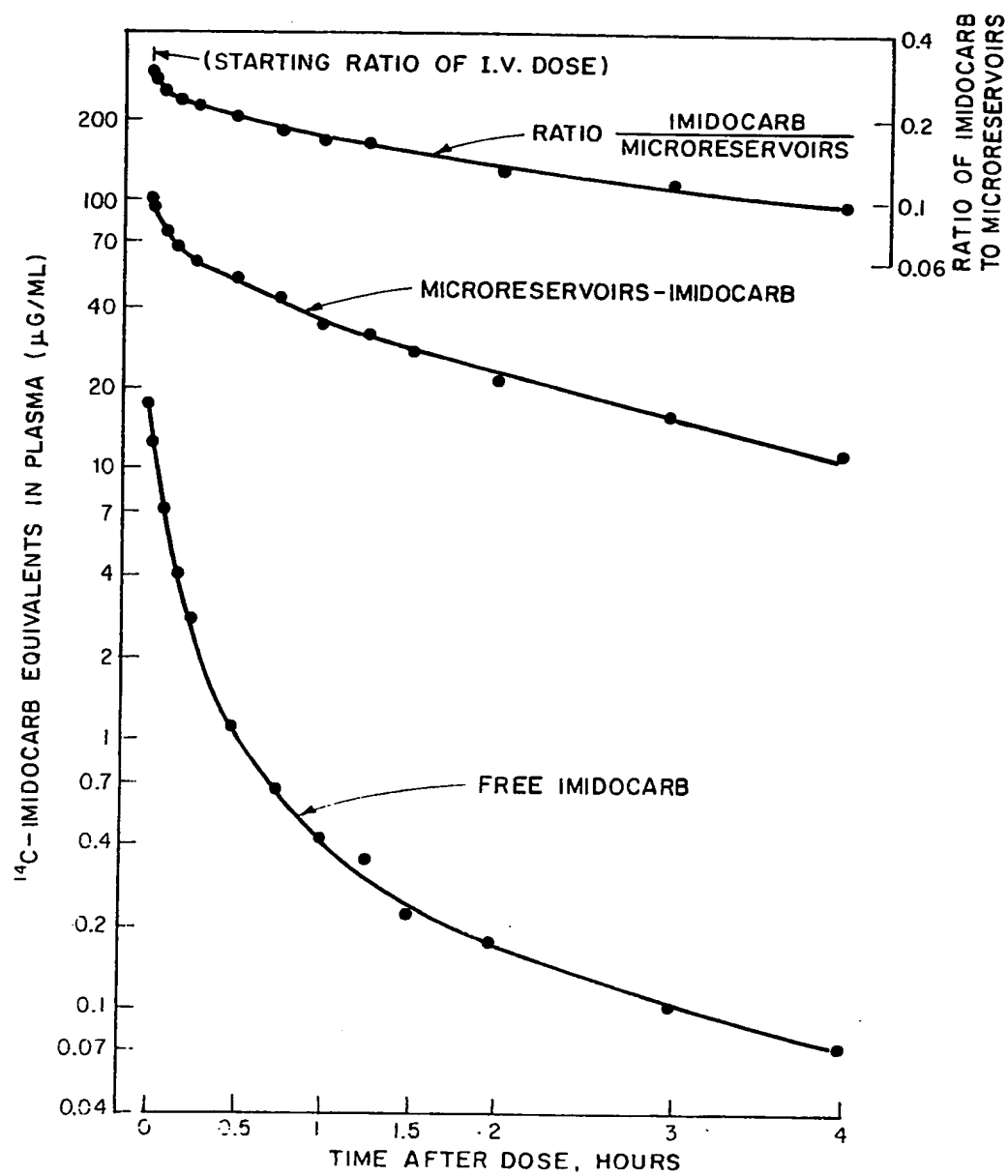
PLASMA KINETICS OF MICRORESERVOIRS-IMIDOCARB
AND FREE IMIDOCARB - DOSE OF 5 MG/KG

Fig. 20
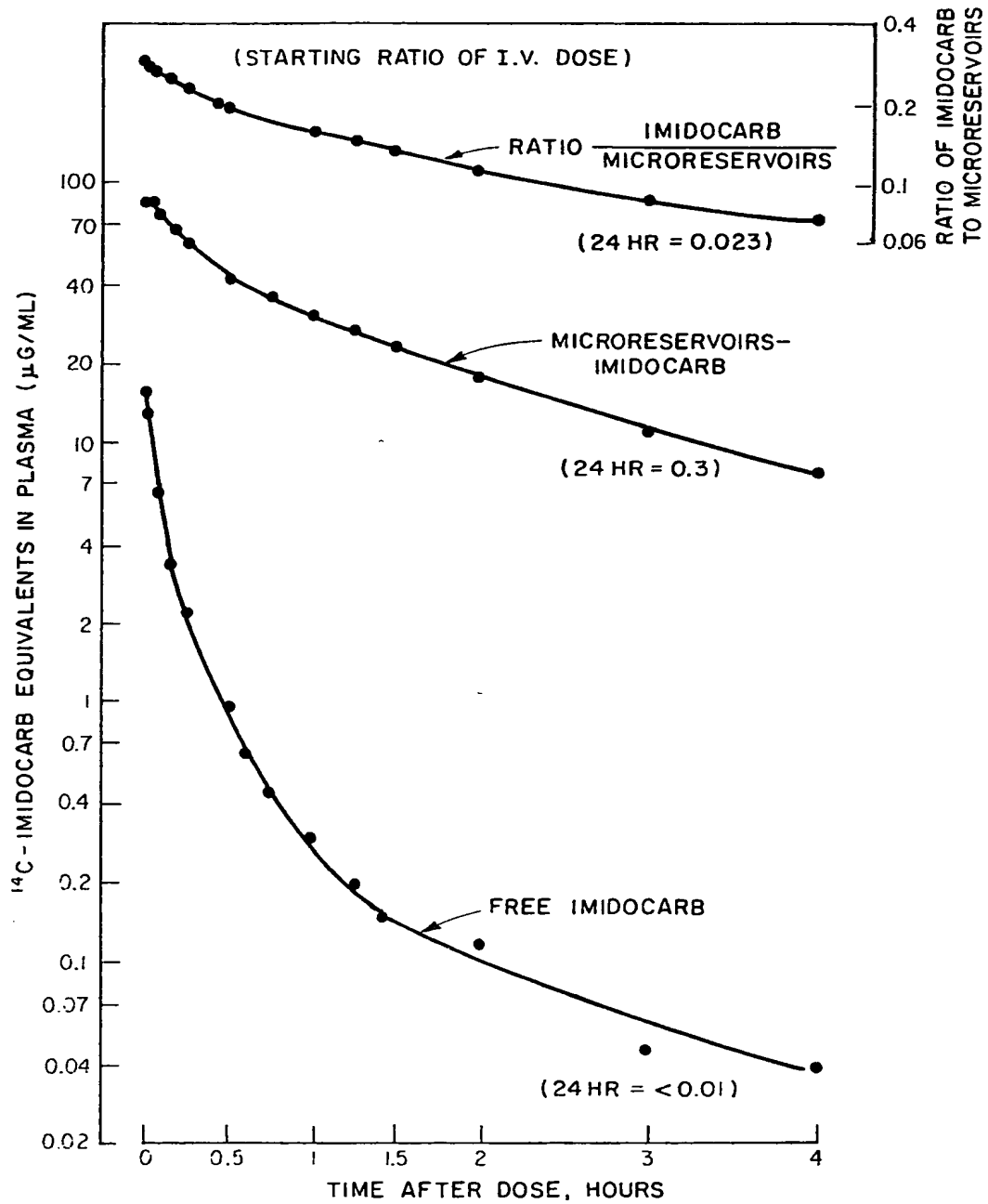
PLASMA KINETICS OF MICRORESERVOIRS – IMIDOCARB
AND FREE IMIDOCARB – DOSE OF 4.4 MG/KG

Fig. 21

ELUTION PROFILE OF MICRORESERVOIRS –
ESTRADIOL UNDECANOATE

Fig.22

CONCENTRATIONS OF
ESTRADIOL UNDECANOATE
IN PLASMA AFTER
ORAL ADMINISTRATION

Fig. 23



EFFLUX RATE OF ESTRADIOL UNDECANOATE FROM
VESICULAR AND NONVESICULAR MICRORESERVOIRS

Fig. 25

ELUTION PROFILE OF VESICULAR AND NONVESICULAR
MICRORESERVOIRS-ESTRADIOL UNDECANOATE
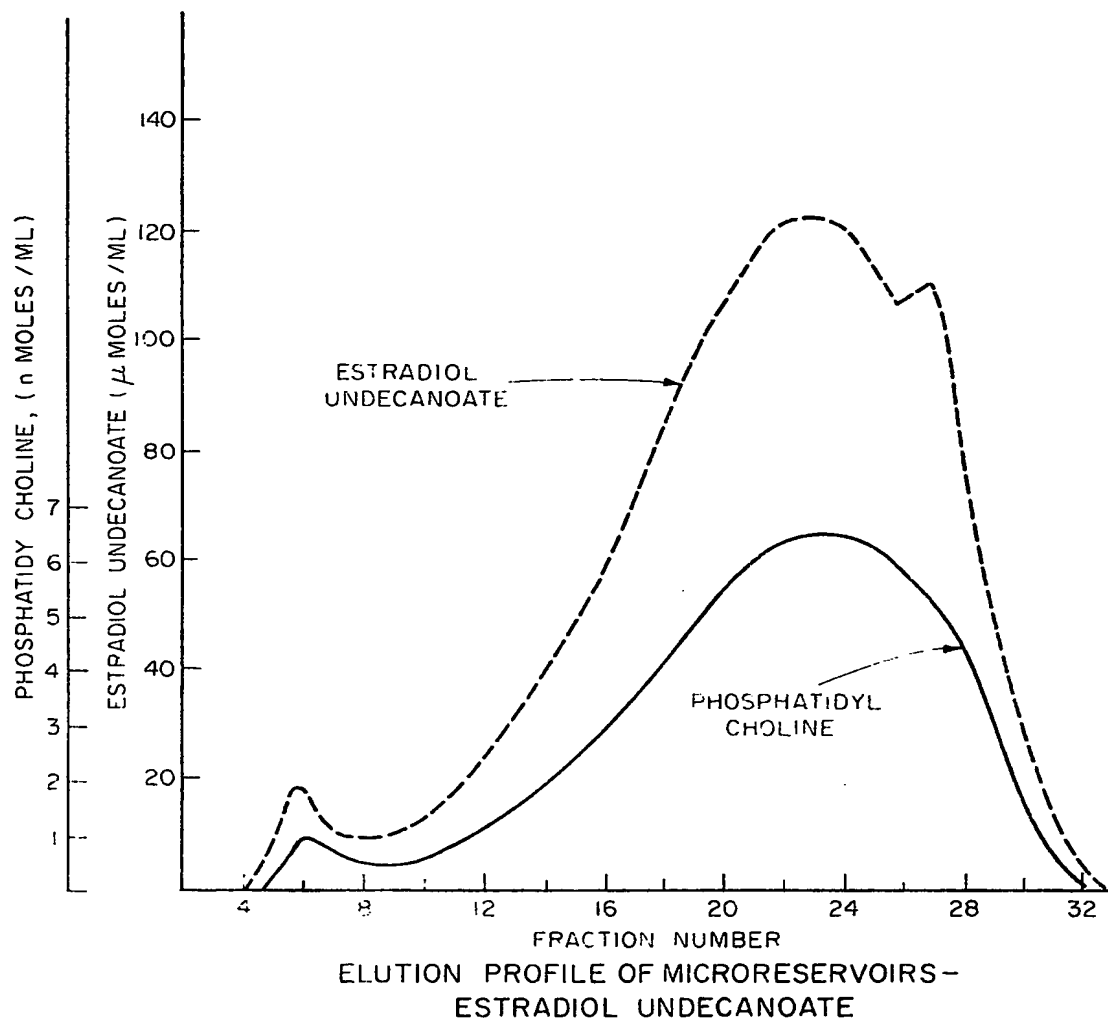
Fig. 24

SPECIFICATION

**Xenobiotic delivery vehicles**

5 This invention relates to the delivery and release of xenobiotics within a mammalian host. More        5
particularly, this invention relates to xenobiotic delivery vehicles in the form of circulating
microreservoirs, to a method of forming the microreservoirs, and to a method of delivering
xenobiotics to a mammalian host which predetermines and controls the pharmacodynamics of
the xenobiotics so delivered and released.

10       In many different situations and under many varied circumstances it is desirable to introduce        10
into a mammalian host pharmacologically active agents which are foreign to the host, these
agents hereinafter being termed "xenobiotics." These xenobiotics include, but are not necessari-
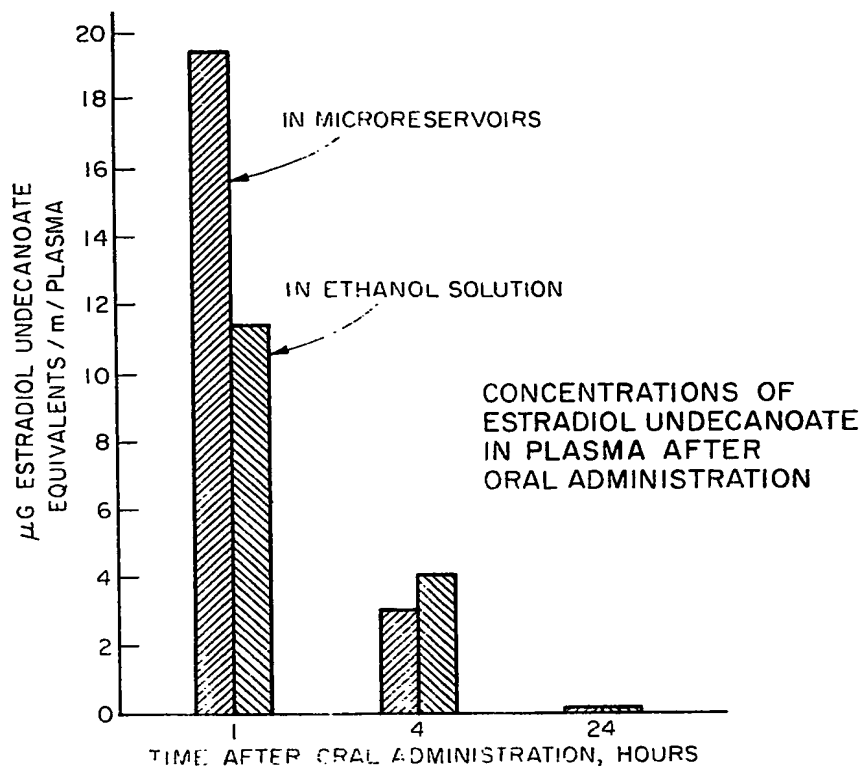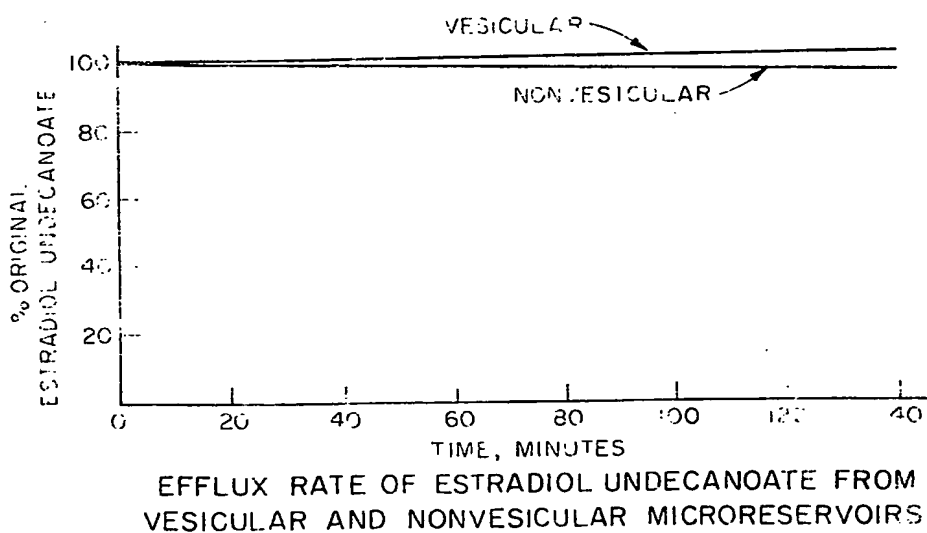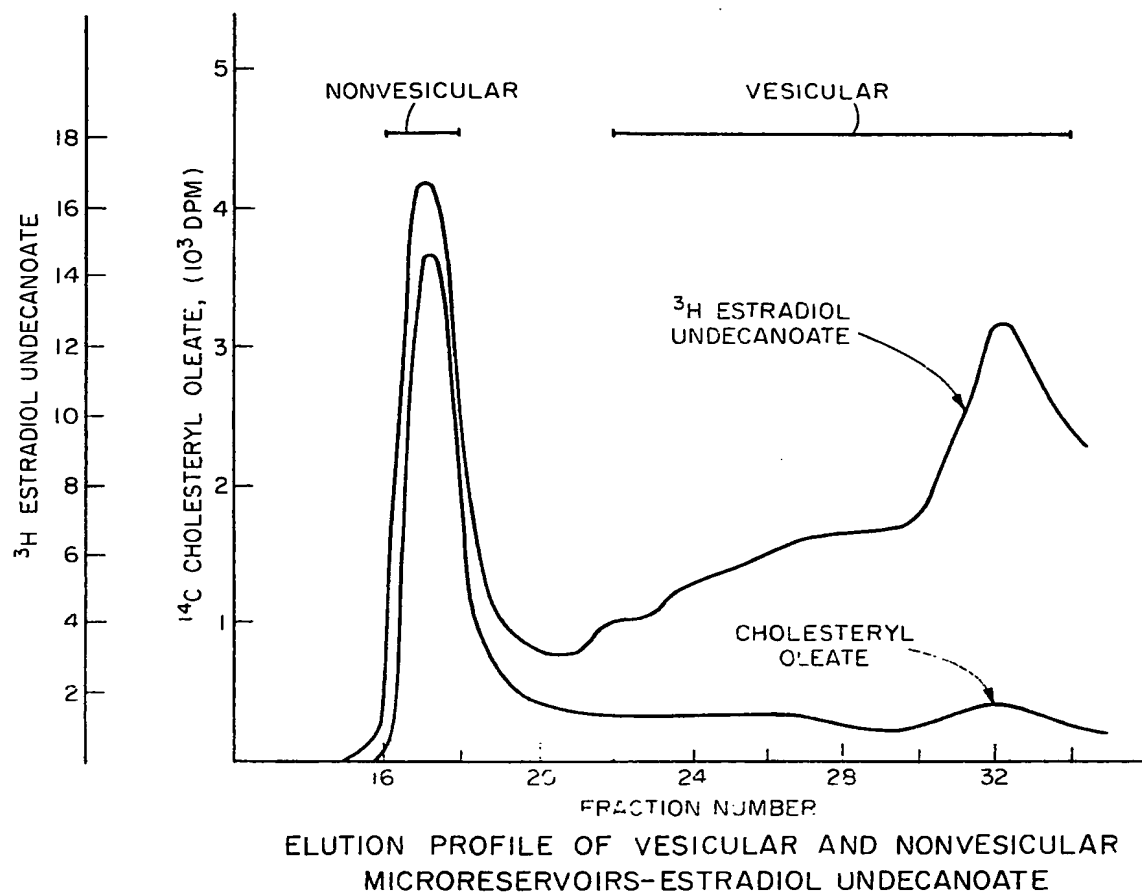ly limited to, drugs, diagnostic agents, blood substitutes, endogenous biological compounds,
hormones, immunological adjuvants and the like.

15       In the administration of any xenobiotic a certain degree of specificity must be attained, and        15
specificity requires that the xenobiotic reach its target selectively and controllably. The absence
of specificity associated with the use of many xenobiotics can thus deprive them of an
appreciable part, if not essentially all, of their potential effectiveness in attaining the results
desired from their use. For example, a chemotherapeutic drug which cannot be retained by

20 blood plasma for a time sufficient for an appreciable amount of the drug to reach the target        20
tissue or an orally administered drug which is destined for the blood stream but which cannot
pass through the gastrointestinal tract lacks the degree of specificity which could make it highly
effective. Thus in lacking the desired specificity, a xenobiotic may exhibit essentially none or
only a limited degree of the pharmacodynamics desired to realize its full potential. Among such

25 pharmacodynamics may be listed plasma kinetics, tissue distribution, degree of toxicity, levels of        25
therapeutic drugs *in vivo*, solubility of xenobiotics normally incompatible with other pharmaceu-
tical formulations, and metabolic activation of the xenobiotics.

         In the prior art it has been recognized that it would be desirable to beneficially alter and
control the specificity or pharmacodynamics of many of the xenobiotics found to have desirable

30 properties. One prior art approach to controlling the specificity of drugs involves the use of        30
implant devices located, normally through a surgical procedure, in or near the organ to which
the drug is to be delivered. Typically, these implant devices comprise a covalent matrix material
containing the drug to be delivered. These matrix materials may be water-soluble (e.g.,
carboxymethyl cellulose or polyvinyl alcohol), water-swellable (e.g., hydrogels or gelatin),

35 hydrolytic polymers (e.g., polyactic acids, polyglycolic acids or poly-$\alpha$-amino acids), or nonhydro-        35
lytic polymers (e.g., organopolysiloxane rubber). Generally, although these implant devices can
control the rate at which the drug they contain can be delivered through diffusion or hydrolysis,
they can exercise little if any alteration of the pharmacodynamics of the drug released.

         One of the more recent approaches suggested for the alteration and control of the

40 pharmacodynamics of xenobiotics is the use of a carrier capable of delivering and controllably        40
releasing the xenobiotics at the site of action within the host to which the xenobiotics are
administered. The use of liposomes has been proposed as carriers for this purpose. (See
Gregoriadis, G., "The Carrier Potential of Liposomes in Biology and Medicines," *The New
England Journal of Medicine*, Vol. 295, No. 13, pp 704–710 [September 23, 1976 and No.

45 14, pp 765–769 [September 30, 1976].) These prior art liposomes are composed of        45
phospholipids, and especially of phsophatidyl choline in combination with other lipids such as
cholesterol, dicetyl phosphate, stearyl amine and other phospholipids such as phosphatidyl
serine with which the phosphatidyl choline is readily miscible. The liposomes are characterized
by the miscibility of the carrier components, a fact which means that there can be little or no

50 phase separation between such components. Furthermore, these carriers lack any high degree of        50
stability, due primarily to the oxidation of the phospholipid components and/or their thermal
instability, especially when they are sonicated to a small size, e.g., about 250 Å. Moreover,
those liposomes which carry a negative charge, due to the presence of dicetyl phosphate,
phosphatidyl serine or other negatively charged components, will aggregate in the presence of

55 divalent cations. Finally, when liposomes are injected intravenously, they are preferentially        55
concentrated in the liver and spleen, due at least in part to their large size. Thus, although the
prior art liposome carriers are compatible with a number of drugs and are biocompatible with
the mammalian system, their inherent instability, tendency to aggregate materially, and
relatively large size detract from their ability to serve as acceptable drug delivery systems.

60       Because of the ever-increasing role played by xenobiotics in the treatment of cancer, diabetes,        60
arthritis, inherited metabolic disorders, metal-storage diseases, and the like, in the prevention of
such diseases as anaplasma and of virus infections, and in the study of biological mechanisms
the need for improved carriers is an ever-growing one. The desirability of having such improved
delivery systems is therefore apparent.

65       It is therefore an object of this invention to provide improved xenobiotic delivery vehicles in        65

the form of microreservoirs capable of circulating within the host system. Another object is to
provide xenobiotic delivery vehicles of the character described which are compatible with a wide
variety of xenobiotics including hydrophobic, hydrophilic or a combination of hydrophobic and
hydrophilic compounds and which are non-toxic and biocompatible with the host system. A

5 further object of this invention is to provide xenobiotic delivery vehicles which are stable over      5
extended periods of storage as well as in their use within the host system and amenable to
various techniques of administration including oral, intravenous, intramuscular, intraperitoneal,
subcutaneous, topical and inhalation.
   Another object of this invention is to provide xenobiotic delivery vehicles capable of

10 predeterminably and beneficially altering and controlling the pharmacodynamics of the xeno-      10
biotic delivered and released within the host system. Among the pharmacodynamics thus
beneficially altered and controlled are plasma kinetics, tissue distribution, toxicity, oral absorp-
tion, chemotherapeutic ability, metabolism and the like.
   It is another object of this invention to provide a method of forming xenobiotic delivery

15 vehicles, in the form of microreservoirs, capable of circulating within a mammalian host thereby      15
to deliver the xenobiotic at a predetermined site by effecting a predetermined beneficial
alteration in the pharmacodynamics of the xenobiotic.
   Yet another object of this invention is to provide a method for delivering and releasing a
pharmaceutically effective amount of a xenobiotic within a mammalian host in a manner to

20 exercise some predeterminable control over the delivery site, thus enhancing the effectiveness of      20
the xenobiotic.
   According to one aspect of this invention there is provided a delivery vehicle biocompatible
with a mammalian host to deliver and release within the host a xenobiotic, the pharmacodynam-
ics of which are beneficially altered through the use of the vehicle, characterized in that the

25 delivery vehicle is in the form of microreservoirs containing the xenobiotic, the microreservoirs      25
comprising a phospholipid constituent and a phospholipid-immiscible lipid constituent stable in
and essentially immiscible with a physiologically-compatible liquid.
   According to another aspect of this invention there is provided a method of forming a
xenobiotic delivery vehicle for delivering within a mammalian host a xenobiotic, the pharmaco-

30 dynamics of which are predeterminably altered and controlled, comprising the steps of forming      30
microreservoirs of a phospholipid constituent and a phospholipid-immiscible lipid constituent
which is stable in and essentially immiscible with a physiologically-compatible liquid; and
incorporating the xenobiotic to be delivered within the microreservoirs.
   According to yet another aspect of this invention there is provided a method of delivering and

35 releasing a xenobiotic within a mammalian host, comprising the step of introducing into the      35
mammalian host a pharmaceutically effective amount of a xenobiotic contained within microres-
ervoirs carried in a physiologically-compatible liquid and formed of a phospholipid constituent
and a phospholipid-immiscible lipid constituent stable in and essentially immiscible with the
physiologically-compatible liquid.

40   According to still another aspect of this invention there is provided a method of predetermin-      40
ing and controlling the pharmacodynamics under which a xenobiotic is delivered within a
mammalian host, comprising the step of releasing the xenobiotic within the host from circulating
microreservoirs formed of a phospholipid constituent and a phospholipid-immiscible lipid
constituent stable in and essentially immiscible with a physiologically-compatible liquid.

45   The invention accordingly comprises the several steps and the relation of one or more such      45
steps with respect to each of the others, and the composition and article possessing the
features, properties, and the relation of constituents, which are exemplified in the following
detailed disclosure, and the scope of the invention will be indicated in the claims.
   For a fuller understanding of the invention, reference should be had to the following detailed

50 description taken in connection with the accompanying drawings, in which:—      50
   Figure 1 is a process flow chart illustrating one method of preparing the xenobiotic delivery
vehicles embodying this invention;
   Figure 2 is a partial process flow chart illustrating a modification of the method of Fig. 1;
   Figure 3 is a much-enlarged diagrammatic representation of a microreservoir embodying this

55 invention in vesicular form;      55
   Figure 4 is a much-enlarged diagrammatic representation of a microreservoir embodying this
invention in nonvesicular form;
   Figure 5 is a much-enlarged diagrammatic representation of a drug delivery vehicle of the
type disclosed in the prior art;

60   Figure 6 illustrates the in vitro stability of the microreservoirs contrasted with that of      60
liposomes, the in vitro stability being measured as the optical density of the microreservoirs and
of the liposomes as a function of time;
   Figure 7 illustrates the in vivo stability of the microreservoirs plotted as the amount of
cholesteryl oleate (cholesterol ester) remaining in the blood plasma of a rat as a function of time;

65   Figure 8 is an elution profile of microreservoirs carying adriamycin plotted as phosphatidyl      65

choline, cholesteryl oleate and adriamycin concentrations in a series of chromatographic fractions;

Figure 9 is a diagrammatic representation illustrating the distribution of a drug such as adriamycin shortly after injecting the free drug, showing how little of the drug remains in the
5 blood stream for delivery to the target site;

Figure 10 illustrates the extent to which the circulating microreservoirs are capable of beneficially altering the plasma kinetics of adriamycin, the illustration being in the form of plots of the amount of adriamycin remaining in the blood-stream as a function of time after injection of the drug in the microreservoirs and as free adriamycin;
10 Figure 11 is a series of plots of the number of survivors of a group of mice as a function of time after the injection at four dose levels of microreservoirs, adriamycin in the microreservoirs and adriamycin in free form, the plots illustrating the ability of the microreservoirs acting as delivery vehicles to decrease the toxicity of the adriamycin;

Figure 12 is a series of plots of the number of survivors of a group of mice injected with
15 tumor cells as a function of time after the injection at two dose levels of adriamycin in the microreservoirs and in free form, the plots illustrating the beneficial altering of the chemotherapy of the adriamycin delivered by and released from the microreservoirs;

Figure 13 comprises a series of plots of efflux rates of adriamycin as a function of time from microreservoirs of compositions containing glycerol trioleate as the phospholipid-immiscible
20 constituent and various amounts of cholesterol as a release-rate control agent;

Figure 14 comprises a series of plots of efflux rates of adriamycin as a function of time from microreservoirs of compositions containing cholesteryl oleate as the phospholipid-immiscible constituent and various amounts of cholesterol as a release-rate control agent;

Figure 15 is an elution profile of microreservoirs carrying AD 32 plotted as phospholipid,
25 cholesteryl oleate and AD 32 concentrations in a series of chromatographed fractions;

Figure 16 is a plot of the efflux rate of AD 32 from vesicular microreservoirs showing the effect of using a minor amount of phosphatidic acid in the phospholipid constituent of the microreservoir composition;

Figure 17 illustrates the extent to which the circulating microreservoirs are capable of
30 beneficially altering the plasma kinetics of AD 32;

Figure 18 is a series of plots of the number of survivors of a group of mice with tumor cells as a function of time after the injection at four dose levels of AD 32 in the microreservoirs, the plots illustrating the chemotherapeutic ability of AD 32 delivered by the microreservoirs;

Figure 19 is an elution profile of microreservoirs carrying imidocarb plotted as phospholipid,
35 cholesteryl oleate and imidocarb concentrations in a series of chromatographed fractions;

Figures 20 and 21 illustrate the extent to which the circulating microreservoirs are capable of beneficially altering the plasma kinetics of imidocarb at two dose levels, the illustrations being in the form of plots of the amount of imidocarb remaining in the bloodstream and the ratios of imidocarb to microreservoirs as functions of time after injection of the drug in the microreser-
40 voirs and as free imidocarb;

Figure 22 is an elution profile of microreservoirs carrying estradiol undecanoate plotted as phosphatidyl choline and estradiol undecanoate concentrations in a series of chromatographed fractions;

Figure 23 is a bar graph showing concentrations in blood plasma at three points in time of
45 estradiol undecanoate given orally as an ethanol solution and in microreservoirs;

Figure 24 is an elution profile of vesicular and non-vesicular microreservoirs carrying estradiol undecanoate; and

Figure 25 is a plot of the efflux rate of estradiol undecanoate from vesicular and nonvesicular microreservoirs.
50 The xenobiotic delivery vehicles embodying this invention are formed of a mixture of two or more lipids which are essentially immiscible. More specifically, the delivery systems are formed of compositions comprising a phospholipid constituent and a phospholipid-immiscible constituent which may be a cholesterol ester or a triglyceride or a mixture of cholesterol esters, or triglycerides or of both these constituents. The phospholipid constituent may comprise more
55 than one phospholipid; and the microreservoir composition may also include a binding modifying and/or a release-rate controlling constituent. Exemplary of phospholipids suitable for the practice of this invention are phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, phosphatidyl ethanolamine, phosphatidic acid and sphingomyelin. Mixtures of two or more of these phospholipids may also be used. Of these, phosphatidyl choline, either alone or in
60 admixture with other phosphalipids, is preferred.

Phosphatidyl choline is a term applied to compounds which are esters of fatty acids with glycerophosphoric acid and choline. Thus phosphatidyl choline may be represented by the following formula:

$$FA-CH_2$$
$$FA'-CHCH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-O-(CH_2)_2-N-(CH_3)_3$$
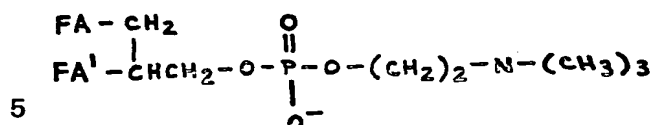
wherein FA and FA' are fatty acid residues. The fatty acids used to form these esters may be saturated or unsaturated and may contain from about 12 to 20 carbon atoms. Such fatty acids
10 include, but are not limited to, palmitic, stearic, oleic and the like. The phosphatidyl choline used in the drug delivery vehicles of this invention may be isolated from egg yolk by the procedure described by Litman (*Biochemistry, 12*:2545[1973]) or from other natural sources such as soybeans or it may be synthesized by a suitable procedure such as that described by Robles and vander Berg (*Biochim Biophys Acta, 187*:520 [1969]).
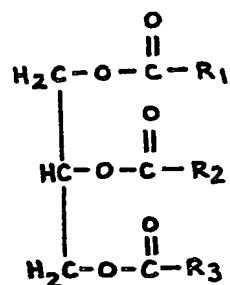15    In the following detailed description of this invention the phospholipid constituent of the delivery vehicles is illustrated, for convenience, by phosphatidyl choline, a term meant to include those esters falling within the general formula given. In some of the microreservoir formulations, minor amounts of phosphatidic acid are incorporated in the phospholipid constituents to improve the binding of the xenobiotic to the microreservoirs. It is also to be understood that
20 other phospholipids, including those named, which meet the chemical and physical properties states below may be used in place of the phosphatidyl choline.
    The cholesterol ester or triglyceride constituent used as a component of the delivery vehicle must be essentially immiscible with the phospholipid constituent as well as essentially insoluble in an aqueous environment as represented by a physiologically-compatible liquid such as a
25 physiologically-balanced salt solution containing NaCl or KCl. The cholesterol ester or triglyceride constituent must also be apolar or nonpolar to the degree that it will not form a monolayer and it must be present in a concentration such that it is essentially immiscible in the phospholipid bilayer.
    Cholesterol, $C_{27}H_{15}OH$, is a monounsaturated, secondary alcohol which readily forms esters
30 with both saturated and unsaturated fatty acids such as oleic, stearic, plamitic and the like. In general, fatty acids having from 10 to 18 carbon atoms are preferred. Suitable procedures for forming these cholesterol esters include the condensation of a fatty acid chloride with cholesterol and the isolation from natural sources.
    In choosing the fatty acid to form the cholesterol ester, it is preferable that it is one with a
35 minor degree of unsaturation (e.g., no greater than about two double bonds per fatty acid). In general, the esters formed of fatty acids with higher degrees of saturation form xenobiotic delivery complexes of greater stability than those formed of highly unsaturated fatty acids.
    The triglycerides suitable for the practice of this invention are fatty acid esters of glycerol having the general formula

$$H_2C-O-\overset{\overset{\displaystyle O}{\|}}{C}-R_1$$
$$HC-O-\overset{\overset{\displaystyle O}{\|}}{C}-R_2$$
$$H_2C-O-\overset{\overset{\displaystyle O}{\|}}{C}-R_3$$

wherein $R_1$, $R_2$ and $R_3$ of the fatty acids forming the esters may have from 10 to 18 carbon atoms. Exemplary of such fatty acids are palmitic, stearic, myristic, oleic and linoleic. These triglycerides are conveniently prepared by the condensation of the fatty acid chloride with
55 glycerol. They may also be isolated from natural sources.
    In the following general description it will be assumed, for convenience, that a cholesterol ester is used. It is, of course, to be understood that a triglyceride, a mixture of cholesterol esters, a mixture of triglycerides or a mixture of one or more cholesterol esters with one or more triglycerides may also be used in forming the microreservoir delivery vehicles. Examples are
60 presented in which either a cholesterol ester or a triglyceride is used as the phospholipid-immiscible constituent.
    The xenobiotic delivery vehicles embodying this invention are in the form of what are hereinafter termed "microreservoirs." These microreservoirs may assume one of two forms, namely a vesicular form which has a small walled cavity containing the medium used in making
65 the microreservoir, or a nonvesicular form which has the cholesterol ester and/or triglyceride

contained within the phospholipid monolayer. As will be described below, the ratio of
phosphatidyl choline to cholesterol ester determines which of these forms predominates in any
synthetic procedure. The choice between these forms is principally dependent upon the nature
of the xenobiotic to be delivered and released, the pharmacodynamics of the xenobiotic desired
5 and the method of administration.                                                                    5
    Two different synthesis routes for forming the microreservoirs, along with the incorporation of
the xenobiotic therein, are illustrated diagrammatically in Figs. 1 and 2, the route shown in Fig.
1 using sonication to form the microreservoirs representing a preferred process. In this process
the phosphatidyl choline and cholesterol ester are mixed in an inert organic liquid which is a
10 solvent for both of these constituents, e.g., chloroform; and, if desired, the xenobiotic is added    10
to this solution as indicated by a dotted line in Fig. 1. The solvent is then removed by
evaporation *in vacuo*, leaving a dry residue mixture which is then hydrated by the addition of a
physiologically-compatible liquid, as exemplified by an aqueous solution of NaCl or KCl of
suitable concentration and a buffer. If the xenobiotic is not added into the initial mixture, it is
15 added into the resulting liquid suspension prior to the formation of the microreservoirs.              15
    In forming the liquid suspension it is preferred to use amounts of the residue mixture
equivalent to between about one and about five percent by weight of the physiologically-
compatible liquid. Although the amount of xenobiotic added to the phospholipid/cholesterol
ester (or triglyceride) composition may vary, it is preferable to incorporate up to about two
20 percent by residue weight of the xenobiotic in the microreservoirs.                                    20
    As illustrated in Fig. 1, the microreservoirs are then formed by sonicating the saline solution
under a non-oxidizing, e.g., nitrogen atmosphere. It has been found that more complete
formation of the microreservoirs occurs when the sonicating is carried out at a temperature
equivalent to or slightly above the melting point of the cholesterol ester or triglyceride serving as
25 the phospholipid-immiscible constituent. The sonication is carried out at a suitable power level    25
and for a time to produce the desired microreservoir size. For example, a power input of 120
watts for 20 minutes has been found satisfactory. As an alternative to the sonicating of the
saline suspension it may be forced through a small orfice to form the microreservoirs.
    As will be seen from Fig. 2, an alternative route to the formation of the microreservoirs in an
30 aqueous medium, e.g., saline solution, lies in the formation of a solution of the phospholipid    30
cholesterol ester and xenobiotic using a water-miscible organic solvent and the subsequent
introduction of this solution into an aqueous physiological saline solution. Alternatively, the
xenobiotic may be added to the saline solution prior to the injecting step. Exemplary of this
procedure is the injection of an ethanol solution of the lipid mixture into a stirred aqueous
35 solution; or the slow injection of an ether solution of the lipid mixture into an aqueous         35
compartment.
    The cloudy liquid resulting from the formation of the microreservoirs in the aqueous
suspending medium is then centrifuged to produce a clear phase and an upper cloudy phase,
the latter containing larger nonvesicular microreservoirs, e.g., from about 300 Å to about 1000
40 Å in diameter. The clear phase is chromatographed to produce a fraction of small (e.g., about     40
250 Å in diameter) nonvesicular microreservoirs and vesicular microreservoirs having diameters
of about 190 Å to about 300 Å.
    The distribution of the microreservoirs between the vesicular and nonvesicular forms is
determined by the mole ratio of phospholipid to phospholipid-immiscible constituent. Using
45 phosphatidyl choline and cholesterol oleate as illustrative of a microreservoir composition, it may   45
be shown that the use of between about 67 and 97 mole % of phosphatidyl choline yields
predominantly vesicular microreservoirs; while the use of more than about 50 mole % of
cholesterol ester yields predominantly nonvesicular microreservoirs.
    The microreservoirs embodying this invention have unique structural properties due to the use
50 of a mixture of the phospholipid constituent and a lipid constituent (cholesterol ester, triglyceride   50
or mixtures thereof) which is essentially immiscible with the phospholipid. This structure, in its
vesicular and nonvesicular forms, is shown diagrammatically in Figs. 3 and 4. The insolubility of
both the phospholipids (represented by phosphatidyl choline in Figs. 3 and 4) and the
immiscible lipid constituent (represented by a cholesterol ester) gives rise to an organized
55 microreservoir structure in which contact between the immiscible lipids and the aqueous          55
environment is avoided or reduced to a minimum. This organization in turn imparts much
greater stability to the microreservoirs than that attained by a vehicle formed in accordance with
the prior art teaching of Gregoriadis previously cited. This may be shown by a comparison of
Fig. 3 and 4 on one hand and Fig. 5 on the other, Fig. 5 representing diagrammatically the
60 structure of the drug delivery system of Gregoriadis. It will be seen from Fig. 5 that when         60
miscible lipids are used to form the liposome the result is a lamellar structure giving rise to an
unstable organization due apparently to the oxidation of the phospholipids which destabilizes the
lamellar organization; or to crystallization of the phospholipids if the temperature decreases
below their transition temperature.
65    The stable structure of the microreservoirs embodying this invention, shown in Figs. 3 and 4,    65

may be attributed, at least in part, to a thermodynamic driving force which prevents the
cholesterol ester or triglyceride from being exposed to the aqueous environment. As a result, the
structural integrity of the microreservoirs embodying this invention is greatly enhanced. This, in
turn, means that the potential for the microreservoirs to control and alter the pharmacodynamics
5  of the xenobiotics incorporated in them is likewise enhanced when compared with other delivery      5
systems. Moreover, the apolarity of the microreservoirs, which is a result of the use of
cholestererol esters or triglycerides in place of cholesterol and the like, essentially prevents
aggregation of the microreservoirs. This is, of course, highly desirable since such aggregation
would otherwise release the cholesterol esters or triglycerides into an aqueous environment with
10  a resulting large decrease in the free energy of the system. Thus, the individual microreservoirs,   10
which are similar in many respects to the naturally occurring circulating serum lipoproteins, are
particularly well adapted to circulate in the blood plasma of the mammalian host into which they
are introduced and to remain in the plasma for maximum effectiveness.
     The greatly enhanced *in vitro* stability of the microreservoirs embodying this invention
15  compared with that of the liposomes suggested in the prior art is illustrated in Example 1 and       15
Fig. 6.

     *EXAMPLE 1*
     Various mixtures of 60 $\mu$moles of egg yolk phosphatidyl choline, various amounts of
20  cholesteryl oleate and 4 mg of adriamycin were taken to dryness under vacuum from a               20
chloroform solution of these microreservoir constituents. To the resulting residue were added 5
ml of 0.1M KCl and 10 mM of trihydroxymethylamine (buffer of pH 8.0); and the resulting
suspension was sonicated at 120 watts for 15 minutes at 51°C under a nitrogen atmosphere.
Both of the suspensions were then spun at 100,000 g for one hour to remove any undispersed
25  lipid.                                                                                              25
     Thin layer chromatograms of the centrifugates showed no evidence of degradation of the
phosphatidyl choline or adriamycin. Aliquots of 4 ml of each sample were then placed into
stoppered curvettes and the samples were incubated at 37°C. At various points in time the
optical densities of the two solutions at 400nm and 600nm were determined. The data thus
30  obtained are plotted in Fig. 6 as the ratio of these optical densities, A400/A600, against time.   30
Since both structures initially had essentially the same molecular size, the ratio of A400/A600
is an indication of any increase in the particle size due to the breakdown of the structural
organization of the liposome.
     Fig. 6 illustrates the dramatic difference in stability between the microreservoirs embodying
35  this invention and similarly-sized liposomes. Whereas the absorbence of the microreservoirs       35
containing 33% cholesteryl oleate, as well as lesser amounts of the cholesterol ester, was
relatively stable, that of the liposome increased rapidly from the beginning of the period of
evaluation and by the end of the 10 days the liposomes had degraded beyond all practical use.
As previously postulated, the marked degradation of the liposomes is believed to be due to the
40  interaction with the aqueous environment and aggregation into larger particles, two factors        40
absent in the microreservoirs by virtue of their structural stability and apolarity.
     In addition to *in vitro* stability, the microreservoirs embodying this invention also exhibit a
greatly increased *in vivo* stability over the liposomes. This is shown in Example 2 and Fig. 7.

45  *EXAMPLE 2*                                                                                         45
     100 $\mu$moles of egg yolk phosphatidyl choline, 10 $\mu$moles of ^{14}C-labeled cholesteryl oleate and
10 $\mu$moles of egg yolk phosphatidic acid were dissolved in chloroform and the solution
evaporated in vacuo to dryness. 5 ml of 0.154 M NaCl and 10 mM of trihydroxymethylamine
(pH 8.0) were added to the dry mixed lipid residue. The resulting suspension was sonicated for
50  15 minutes at 51°C under a nitrogen atmosphere. The sonicated mixture was then chromato-         50
graphed on a 2.5 X 40cm Sepharose 4B column. Individual fractions that demonstrated a
coincidence in the elution profile of the phospholipids and cholesteryl oleate were pooled and
then concentrated by ultrafiltration. 1.1 ml of these concentrated microreservoirs were injected
into the tail vein of a rat. Plasma samples were withdrawn at various time periods and assayed
55  for the radioactivity associated with the microreservoirs contained in the plasma sample. The     55
data thus obtained are plotted in Fig. 7 as a function of time after injection.
     Although the kinetics are complex, it can be seen from Fig. 7 that at equilibrium, the
clearance of the microreservoirs has a half-life of 5.5 hours. In contrast, sonicated liposomes
containing a similar negative charge have a plasma half-life of only 8 minutes. (R.L. Juliano and
60  D. Stamp, *Biochem Biophys. Res Comm 63*: 651 [1975].) It seems logical to postulate that the     60
nearly 40-fold increase in the lifetime achieved by the microreservoirs is attributable to increased
structural stability.
     The association of a xenobiotic incorporated in the microreservoirs is illustrated in Example 3
and Fig. 8.

*EXAMPLE 3*

2180 μmoles of egg yolk phosphatidyl choline, 242 μmoles of cholesteryl oleate labeled with
$^{14}$C (specific activity $1.6 \times 10^4$ disintegrations per minute (dpm)/μmole and 36 mg of adriamy-
cin were mixed together in chloroform and the solution was evaporated to dryness *in vacuo.* The
5  mixed dry residue was then hydrated by the addition of 110 ml of 0.1M KCl and 10 mM of               5
trihydroxymethylamine (pH 8.0). This suspension was then sonicated with a Branson W-185
Sonifer for 15 minutes at 51°C under a nitrogen atmosphere.
The sonicated liquid was then chromatographed on a $2.5 \times 40$ cm Sepharose 4B column.
Individual fractions resulting from the chromatographing were then assayed for phosphatidyl
10  choline content by the procedure of Gomori (*J. Lab. Clin. Med, 27*: 955 [1949]), for cholesteryl   10
oleate by radioactivity and for adriamycin by fluorescence measurements. The results of these
analyses are plotted in Fig. 8 with the analytical results for the cholesteryl oleate, phosphatidyl
choline and adriamycin being superimposed.
The diameter of the vesicular microreservoirs was found to be approximately 200 Å to 300 Å
15  as determined by their elution profile on the gel column which had previously been calibrated       15
using sonicated liposomes of egg yolk phosphatidyl choline.
It can be seen from Fig. 8 that the elution profiles of the phosphatidyl choline (as monitored
by phosphate analysis), of the cholesteryl oleate (as monitored by radioactivity) and of the
adriamycin (as monitored by fluorescence) coincide, indicating that the xenobiotic (adriamycin)
20  was associated with the vesicular microreservoirs. Since adriamycin is a relatively hydrophobic    20
drug, with some hydrophilic characteristics, it is reasonable to postulate that the site of
localization of the drug is within the lipid organization of the microreservoirs.
Among the pharmacodynamics of a xenobiotic which can be altered to make the xenobiotic
more effective are plasma kinetics, degree of toxicity and therapeutical effectiveness. Adriamycin
25  is considered to be one of the more effective cancer chemotherapeutic drugs; but it is relatively    25
hydrophobic and it is very difficult to maintain in the plasma stream as evidenced by the fact
that within two minutes after administration about 95% of it has left the bloodstream and gone
to various organs leaving only some 5% to reach the target tissue, i.e., a tumor. This is
illustrated in Fig. 9 which illustrates the distribution of adriamycin, given intravenously, minutes
30  after injection. Finally, adriamycin is known to be toxic, particularly since it tends to concentrate  30
in the heart tissues, making it necessary not only to carefully monitor its administration but to
administer it at very low dosage levels.
The following Examples 4–6 and Figs. 10–12 illustrate the ability of the microreservoirs
embodying this invention circulating in the bloodstream to alter the plasma kinetics, toxicity and
35  chemotherapeutical effectiveness of adriamycin.                                                      35

*EXAMPLE 4*

200 μmoles of egg yolk phosphatidyl choline, 20 μmoles of cholesteryl oleate and 4 mg of
adriamycin were dissolved in chloroform and then taken to dryness under vacuum. To the
40  resulting dry residue were then added 5 ml of 0.154 M NaCl and 10 mM of trihydroxymethy-       40
lamine (pH 7.4). This aqueous suspension was then sonicated for 15 minutes at 51°C under a
nitrogen atmosphere. The adriamycin which was not contained within the vesicular microreser-
voirs formed was separated from the microreservoirs by passage through a $2.5 \times 20$ cm
Sephadex G-50 gel column. The void volume which contained the adriamycin incorporated in
45  the microreservoirs was concentrated to 3 mg adriamycin/ml.                                   45
A control sample of free adriamycin at the same concentration and in the same buffer was
also prepared.
Three rats were injected intravenously with the microreservoirs containing adriamycin; and six
other rats were injected intravenously with the free adriamycin. The dosage of the adriamycin
50  contained within the microreservoirs was 4 mg/kg; and that for the free adriamycin was 4       50
mg/kg in three rats and 10 mg/kg in the remaining three rats. 0.5 ml of blood was taken at
various time points from each rat. The plasma was separated from the blood samples and
assayed for adriamycin fusing fluorescence. The data thus obtained are plotted in Fig. 10 as a
function of time after injection.
55  It is apparent from Fig. 10 that the use of the circulating microreservoirs as a delivery vehicle  55
for the adriamycin beneficially alters and improves the plasma kinetics for that drug. From a
comparison of Curves A and B, representing the same dosage level, it will be seen that after
about 15 minutes the concentration of adriamycin in the circulating microreservoirs in the
plasma was about 4 μg/ml; while the concentration of the free adriamycin in the plasma was
60  about 0.4 μg/ml. Therefore, at this time point, the concentration of adriamycin in the          60
bloodstream carried by the circulating microreservoirs was some ten times greater than when
this drug was introduced directly into the bloodstream. Furthermore, as will be seen from Fig.
10, the free adriamycin was virtually absent from the bloodstream after some 3 3/4 hours;
while it was still up to a concentration of about 0.4 μg/ml in the circulating microreservoirs at
65  this time point. Thus the concentration of the drug in the bloodstream carried in the circulating  65

microreservoirs after 3 3/4 hours was equivalent to that after only 15 minutes when the drug
was introduced in the free form.
    A further comparison of Curve A with Curve C (free adriamycin in a dosage of 10 mg/kg)
shows that after one hour the concentration of the drug in the plasma was about 1.3 μg/ml
5 when carried by the circulating microreservoirs and about 0.5 μg/ml when in free form; after 3        5
hours these figures were about 0.5 μg/ml and about 0.3 μg/ml; and after 6 hours they were
about 0.21 μg/ml and about 0.14 μg/ml, respectively. Thus, the use of the circulating
microreservoirs embodying this invention can provide concentrations of this drug in the
bloodstream which are materially greater than those achieved when the same drug is
10 administered in free form in dosages 2.5 times greater than when administered through the        10
microreservoirs.
    The altering of the plasma kinetics of a drug such as adriamycin, and as illustrated in Fig. 10,
means that it has a less toxic effect since it is maintained within the blood-stream longer and is
thus prevented from concentrating in such organs as the liver, kidney and heart. It also means
15 that any given dosage is more effective when carried by the circulating microreservoirs than        15
when circulating freely, since the more drug that remains in the bloodstream the more exposure
there will be of the target tissue to the adriamycin dose. Since the effectiveness of this drug in
killing tumor cells is based upon the ability of the tumor cells to take it up, maximum contact
between the drug and cells is essential for maximum effectiveness.
20    Finally, altering of the plasma kinetics of a xenobiotic as shown in Fig. 10 offers the        20
possibility of decreasing dosages; for if the performance of, say, the 10 mg/kg dose of Curve C
could be considered adequate for chemotherapeutical purposes, it becomes apparent that the
dosage level could be reduced below 4 mg/kg if the drug is delivered and released from the
circulating microreservoirs. Such a decrease in dosage would also materially reduce toxicity.
25    That a marked reduction in adriamycin toxicity is achieved through the use of the circulating        25
microreservoirs is shown in Example 5 and Fig. 11.


    EXAMPLE 5
    1000 μmoles of egg yolk phosphatidyl choline, 100 μmoles of cholesteryl oleate and 20 mg
30 of adriamycin were dissolved in chloroform and the solution was taken to dryness in vacuo. To        30
the resulting dry residue mixture were added 20 ml of 0.1 M KCl and 10 mM of
trihydroxymethylamine (pH 8.0). The aqueous suspension was sonicated for 15 minutes at 51°C
under a nitrogen atmosphere. The sonicated liquid was chromatographed on a 2.5 × 40 cm
Sepharose 4B gel column. Only those fractions which showed a coincidence of the elution
35 profile of phsophatidyl choline, cholesteryl oleate and adriamycin were pooled. These pooled        35
fractions were then concentrated by ultrafiltration, using an XM-50 membrane, to a final
concentration of 3 mg of adriamycin per ml.
    A similar sample of microreservoirs without adriamycin was also prepared and concentrated to
the same microreservoir concentration. A solution of free adriamycin at a concentration of 3
40 mg/ml in 0.1 M KCl and 10 mM of trihydroxymethylamine (pH 8.0) was also prepared as a        40
control to be used as free adriamycin.
    The microreservoirs containing the adriamycin, the microreservoirs without the drug, and the
free drug were injected at various drug doses into mice, using a single intraperitoneal injection.
Ten BDF₁ mice were used for each protocol; and the resulting data are plotted in Fig. 11. These
45 plots show the survival rate of the mice as a function of time. It will be seen that the        45
microreservoirs themselves, in the absence of adriamycin, exhibited no toxicity. At all of the
dosage levels, the use of the circulating microreservoirs to carry the adriamycin decreased the
toxic effects of the drug compared to that exhibited by the free adriamycin. As the dosage levels
decreased the ability of the delivery vehicles to reduce toxicity became more marked in
50 comparison with the free drug. It is believed that this reduction in toxicity can be explained, at        50
least in part, by the ability of the circulating microreservoirs to keep the adriamycin the in the
blood-stream and out of the tissues and organs, particularly the heart.
    The effect of the chemotherapy of adriamycin using the delivery vehicles of this invention is
illustrated in Example 6 and Fig. 12.
55                                                                                                        55
    EXAMPLE 6
    The procedures of Example 5 were followed to make vesicular microreservoirs with and
without adriamycin and to prepare adriamycin for use as a free drug. A number of mice were
injected intraperitoneally with 1 × 10⁶ P388 tumor cells. Twenty-four hours later single daily
60 intraperitoneal doses of the vesicular microreservoirs with and without adriamycin and free        60
adriamycin were injected for a period of 5 days after the tumor implants. One group of mice,
used as a control, received no drug in any form. Five mice were used for each protocol and two
different dosage levels, 4 mg/kg and 2 mg/kg, were used. The data obtained from these tests
are plotted in Fig. 12 as number of survivors as a function of time.
65    The mice injected with the microreservoirs without adriamycin had a survival rate (not plotted)  65

similar to that of the control mice, Curves C in the plots. At the dosage level of 4 mg/kg the
adriamycin delivered by the circulating microreservoirs exhibited chemotherapeutic effect while
the free drug at the same dosage level demonstrated a toxicity measurably greater than the
tumor cells. At the lower dosage level of 2 mg/kg, the adriamycin delivered by the circulating

5     microreservoirs exhibited a greater chemotherapeutic response than the free drug.                                5
    In formulating the microreservoirs embodying this invention, it may be desirable to include
one or more xenobiotic binding modifiers which are capable of either increasing or decreasing
the relative amount of the xenobiotic picked up by the microreservoirs during their formation.
For example, it has been found that a minor mole percent, i.e., about 5 mole percent or more,

10    of phosphatidic acid, a charged lipid, added to the phospholip constituent of the microreservoirs   10
may increase the affinity of the xenobiotic for the microreservoirs. It is, therefore, within the
scope of this invention to use one or more different phospholipids to make up the phospholipid
constituent of the microreservoir composition. It is, moreover, within the skill of the art to
choose a single phospholipid or an optimum combination of phospholipids as the phospholipid

15    constituent of the microreservoirs to obtain a predetermined pickup of the xenobiotic.                            15
    It is also possible to modify the binding of a xenobiotic to the reservoir by the inclusion of
other lipids which are soluble in the phospholipid constituent or slightly soluble in the
cholesterol ester or triglyceride constituent. The modifications achieved in a microreservoir/adri-
amycin system are illustrated in Example 7 and Table 1; and the effect such modifications have

20    on the xenobiotic efflux or release rates of the resulting microreservoirs is illustrated in Example   20
8 and Figs. 13 and 14.

*EXAMPLE 7*
    A series of formulations of microreservoirs containing 105 $\mu$mole of $^{14}$C-labeled egg yolk

25    phosphatidyl choline (specific activity 4160 dpm/$\mu$mole phosphatidyl choline), 11.6 $\mu$moles of   25
cholesteryl oleate, or 11.6 $\mu$moles of triolein (glyceryl trioleate), with or without a second
phospholipid as represented by 11.6 $\mu$moles of phosphatidic acid, and with or without
cholesterol as a modifying agent present in an amount from 0 to 75 $\mu$moles was made up. In
each formulation, 1.72 mg of adriamycin was used as the xenobiotic to be carried and released.

30    The formulations were prepared as solutions of chloroform which were taken to dryness in 10-   30
ml screw-cap vials and pumped overnight. Each formulation sample was then hydrated with 3
ml of 0.154 M NaCl and 10 mM of trihydroxymethylamine (pH 7.2) and then vortexed for
several minutes at room temperature. Each sample was then sonicated at the appropriate
temperature for 20 minutes in a stream of nitrogen. Sonication of those samples containing

35    cholesteryl oleate was carried out at 51°C; and of those containing triolein at 3°C. After   35
sonication, each sample was spun in a desktop centrifuge for 10 minutes to remove any
titanium fragments.
    Each sample was passed down a 2.5 × 15 cm Sephadex G-50 column using 0.154 M NaCl
and 10 mM trihydroxymethylamine as the eluting buffer. The void volume of each of the G-50

40    columns, which contained the microreservoirs, was collected and assayed for $^{14}$C egg yok   40
phosphatidyl choline radioactivity and for the fluorescence from the adriamycin. The results of
these measurements in terms of $\mu$g adriamycin/$\mu$mole phosphatidyl choline and $\mu$g adriamy-
cin/$\mu$mole total phospholipid and the percent of adriamycin pick up by the microreservoirs are
tabulated in Table 1.

45    In reporting these results, the percent adriamycin encapsulated was normalized to the sample   45
that had the highest degree of encapsulated adriamycin per $\mu$mole of total phospholipid.

TABLE 1
EFFECT OF COMPOSITION OF PHOSPHOLIPID CONSTITUENT AND OF THE ADDITION OF
PHOSPHOLIPID-MISCIBLE LIPIDS ON THE PICKUP OF ADRIAMYCIN BY MICRORESERVOIRS

| Sample No. | Microreservoir Composition Mole% | | | | | μg Adriamycin/ | | |
|---|---|---|---|---|---|---|---|---|
| | PC | GTO | CO | PA | Chol | μmole PC | μmole PL | % Encap- sulated |
| 1 | 82 | 9 | — | 9 | — | 6.67 | 6.03 | 100 |
| 2 | 90 | 10 | — | — | — | 4.90 | 4.90 | 81 |
| 3 | 82 | 9 | — | — | 9 | 4.26 | 4.26 | 71 |
| 4 | 72 | 8 | — | — | 20 | 4.14 | 4.14 | 69 |
| 5 | 65 | 7 | — | — | 28 | 4.03 | 4.03 | 67 |
| 6 | 54 | 6 | — | | 40 | 3.91 | 3.91 | 65 |
| 7 | 82 | — | — | 9 | — | 5.83 | 5.27 | 87 |
| 8 | 90 | — | 10 | — | — | 4.41 | 4.41 | 73 |
| 9 | 82 | — | 9 | — | 9 | 4.21 | 4.21 | 70 |
| 10 | 72 | — | 8 | — | 20 | 3.43 | 3.43 | 57 |
| 11 | 65 | — | 7 | — | 28 | 3.14 | 3.14 | 52 |
| 12 | 54 | — | 6 | — | 40 | 2.36 | 2.36 | 40 |

PC——Phosphatidyl choline
GTO——Glycerol trioleate
CO——Cholesteryl oleate
PA——Phosphatidic acid
Chol——Cholesterol
PL——Total phospholipids (PC and PA)

From the data in Table 1 it will be seen that the inclusion of a small amount of phosphatidic
acid in the phospholipid constituent (Samples 1 and 7) increased the uptake of adriamycin by
the microreservoirs. In contrast to imidocarb, (Examples 13–15), adriamycin is an uncharged
molecule, a fact which indicates that the use of phosphatidic acid as a portion of the
phospholipid constituent is applicable to xenobiotics of varying characteristics.

The incorporation of cholesterol, a phospholipid-miscible lipid, in the microreservoir composi-
tion inhibited the binding of the adriamycin to the microreservoirs. The addition of cholesterol to
microreservoir compositions containing glycerol trioleate (Samples 3–6) had a markedly less
effect on the binding of the drug than in the case where the microreservoir composition
contained cholesteryl oleate (Samples 9–12). Finally, the data of Table 1 indicate that in the
case of adriamycin the use of glycerol trioleate (Samples 1–6) in place of cholesteryl oleate
(Samples 7–12) facilitates the binding of this xenobiotic to the microreservoirs. It is therefore
apparent that through the choice of the phospholipid constituent and the phospholipic-
immiscible constituent (with or without an additional xenobiotic binding modifier) it is possible
to control and predetermine the degree of xenobiotic uptake in or binding to the microreservoirs.
Such control offers flexibility in the xenobiotic delivery system of this invention with respect to
dosage levels, rate of xenobiotic release, and the like.

Not only can the equilibrium binding of a xenobiotic be affected by the microreservoir
composition, but also the efflux or release rate of the xenobiotic can be controlled and
predetermined by the composition. This is illustrated through the use of a model system which
permitted the determination of xenobiotic efflux rates from the microreservoirs in response to
nonequilibrium conditions as detailed in Example 8 and shown in Figs. 13 and 14.

EXAMPLE 8
Microreservoirs were formulated as described in Example 7. To establish the required
nonequilibrium conditions the microreservoirs containing adriamycin as the xenobiotic were
incubated with a large excess of unsonicated egg yolk phosphatidyl choline dispersions. These
dispersions were formed by adding aliquots of microreservoirs of the various compositions
containing approximately 1 μmole of egg yolk phosphatidyl choline to 9 mmoles of unsonicated
egg yolk phosphatidyl choline in 1.0 ml of 0.154 M NaCl and 10 mM of trihydroxymethylam-
ine (pH 7.2). Comparable control dispersions were made up to contain an equivalent amount of
free adriamycin in place of that carried by the microreservoirs.
In assessing the efflux rate of the adriamycin, one of the mixtures thus formed was used for

each time point. At the designated time, the sample was centrifuged at 15,000g for 2 minutes
under which conditions the unsonicated dispersion was readily separated from the microreser-
voirs remaining in the resulting supernatant. Since the adriamycin tends to re-equilibrate
between the microreservoirs and the unsonicated phospholipid dispersions, the rate of equilibri-
5  um can be used as a kinetic parameter to evaluate the role of various constituents forming the                    5
microreservoir composition in determining the efflux rate of the adriamycin contained in the
microreservoirs.
      An aliquot of the resulting supernatant was assayed for fluorescence and the amount of
fluorescence thus remaining was compared with that initially present in the mixture. This
10  amount of adriamycin remaining in the supernatant therefore represents the amount of the drug    10
still contained in the microreservoirs.
      The data obtained from this series of measurements are plotted in Figs. 13 and 14 which
show descrease of fluorescence as a function of time for the microreservoirs containing glycerol
trioleate (Fig. 13) and cholesteryl oleate (Fig. 14). From the data plotted in Figs. 13 and 14 it
15  will be seen that the free adriamycin was very rapidly removed from the supernatant, whereas    15
that bound to the microreservoirs was retained to a much greater degree. The inclusion of 9
mole % of phosphatidic acid in the phospholipid constituent of the microreservoirs materially
retarded the efflux rate, while the addition of cholesterol increased it. Finally, the use of
triglyceride in place of a cholesterol ester slightly decreased the efflux rate.
20      These kinetic evaluations based on efflux rates contribute to the significance of the data of    20
Table 1 concerning the equilibrium binding of adriamycin to the microreservoirs; and these data
confirm the fact that the composition of the microreservoirs can be chosen to predetermine and
control the pharmacodynamics of the xenobiotic contained in the microreservoirs serving as the
drug delivery vehicle of this invention.
25      AD 32 is a highly hydrophobic analog of adriamycin used in chemotherapy. Since this drug is    25
totally insoluble in an aqueous buffer, it is necessary to use a combination of a detergent and an
organic liquid to form a solution of AD 32 suitable for clinical use. Exemplary of one such
solvent presently in use is a mixture of equal volumes of ethanol and a sulfated ethylene glycol
detergent (emulphol).
30      In spite of the hydrophobic nature of AD 32, it is possible to incorporate it into the    30
microreservoirs embodying this invention and thus to beneficially alter its pharmacodynamics
with respect to both plasma kinetics and therapeutic effectiveness as illustrated in Examples
9–12 and Figs. 15–18.

35  *EXAMPLE 9*    35
      1090 $\mu$moles of phosphatidyl choline derived from egg yolk, 121 $\mu$moles of ^{3}H-labeled
cholesteryl oleate (specific activity of 190,000 dpm/$\mu$mole) and 17.5 mg of AD 32 were
dissolved in chloroform, then taken to dryness and pumped overnight. 5.5 ml of 0.154 M NaCl
and 5 mM of trihydroxymethylamine (pH 7.41) were added to the dry mixture. The liquid was
40  sonicated at 48°C under a nitrogen atmosphere and the resulting sonicated liquid was    40
chromatographed on a 2.5 × 40 cm Sepharose 4B column. Individual fractions thus obtained
were assayed for phosphatidyl choline content, for cholesteryl oleate and for AD 32 as
previously described. The elution profile, a composite of the assays, is plotted in Fig. 15. From
this plot it can be seen that the AD 32 is associated with both nonvesicular microreservoirs
45  (fractions 2–5) and vesicular microreservoirs (fractions 6–18).    45
      The inclusion of a minor amount of phosphatidic acid in the phospholipid constituent of the
microreservoir composition was found to have little, if any, effect on the efflux rate of AD 32
when a triglyceride was used as the phospholipid-immiscible constituent. This is evident from
Example 10 and Fig. 16.
50                                                                                                              50
*EXAMPLE 10*
      A basic microreservoir formulation containing 104.6 $\mu$moles egg yolk phosphatidyl choline,
and 11.6 $\mu$moles of ^{3}H-labelled glycerol trioleate (specific activity of 1.5 × 10^{5} dpm/$\mu$mole) was
used. 1.73 mg of AD 32 was added and in one formulation 11.62 $\mu$moles of egg yolk
55  phosphatidic acid was also added. These formulations were taken to dryness from a chloroform    55
solution and pumped overnight under vacuum. 3 ml of 0.154 M NaCl and 10 mM
trihydroxymethylamine were added to each sample and the hydrated liquids were sonicated for
20 minutes at 3°C under a nitrogen atmosphere. Each sample was passed down a 2.5 × 20 cm
Sephadex G-50 column and a quantity of each resulting vesicular microreservoir sample
60  equivalent to one $\mu$mole of phospholipid was incubated with 9 $\mu$moles of phosphatidyl choline    60
liposomes in 0.46 ml of a buffered saline solution to form a dispersion.
      At various time points, the resulting dispersions were centrifuged at 15,000g for 2 minutes
and the amount of fluorescence remaining in the supernatant was determined. The data from
these measurements of efflux rate are plotted in Fig. 16. It will be seen that the efflux rate of
65  AD 32 is relatively rapid and essentially unaffected by the inclusion of phosphatidic acid in the    65

microreservoir composition.

Although the efflux rate of AD 32 from the microreservoirs is comparatively rapid, the use of microreservoirs bring about a marked beneficial alteration of the plasma kinetics of this drug, compared to the presently used dosage forms. This is illustrated in Example 11 and Fig. 17.

*EXAMPLE 11*

Fractions 6–18 of Example 9 were concentrated by ultrafiltration to an AD 32 concentration of 3.2 mg/ml. Sufficient quantities of the AD 32 in the microreservoirs were injected intravenously into 150-gram rats to provide a dosage level of 15 mg/kg; and a clinical formulation of AD 32 dissolved in a 1 to 1 by volume mixture of ethanol/emulphol was similarly injected at the same dosage level in three control rats. Plasma samples were taken at various points in time and AD 32 concentrations in these plasma samples were determined by fluorescence. The resulting data (average of three rats for each point) are plotted in Fig. 17 as AD 32 equivalents as a function of time from initial injection.

Fig. 17 shows the marked alteration in the plasma kinetics of AD 32 achieved through the use of the microreservoirs embodying this invention, for the levels of AD 32 in the plasma in which the drug was incorporated in microreservoirs were consistently higher by a factor of between 2 and 3 at all time points than for the free AD 32 introduced as the clinical solution. By allowing the drug a longer period of time to seek out tumor cells, this alteration in its plasma kinetics enhances its effectiveness as a chemotherapeutic agent. This is shown in Example 12 and Fig. 18.

*EXAMPLE 12*

Vesicular microreservoirs containing AD 32 were formulated as in Example 9 and concentrated to 3.2 mg AD 32/ml. A number of mice were injected intraperitoneally with $1 \times 10^6$ P388 tumor (leukemic) cells. Twenty-four hours later single daily intraperitoneal doses of the vesicular microreservoirs with AD 32 and saline control solutions were injected for a period of 5 days after the tumor implants. Five mice were used for each protocol and four different dosage levels, 7.3 mg/kg, 4 mg/kg, 2 mg/kg, and 1 mg/kg were used. The data obtained from these tests are plotted in Fig. 18 as number of survivors as a function of time. In all cases the mice injected with the microreservoirs containing AD 32 had a survival rate higher than that of the control mice. Moreover, even at the very low dosage level of 1 mg/kg the AD 32 delivered by the circulating microreservoirs exhibited chemotherapeutic effect and brought about a significant increase in survival of mice given the P388 leukemic cells.

As in the case of adriamycin, the same plasma concentrations of AD 32 can be achieved with lower initial levels of AD 32 carried by the microreservoirs than in clinical formulations. Furthermore, the microreservoir composition is more biocompatible with blood than the mixed solvent of ethanol and detergent now required in clinical formulations of AD 32.

Adriamycin and AD 32, as well as carminomycin, are cancer chemotherapeutic agents falling within the general class of anthracycline drugs. The preceding examples illustrate the effectiveness of the delivery vehicle embodying this invention in beneficially altering the pharmacodynamics of this class of chemotherspeutic agents. The microreservoirs embodying this invention may also be used to deliver other classes of chemotherapeutic agents including, for example, nitrosoureas and metabolites.

Imidocarb is a drug which has proven very effective as a parasiticide in the treatment of anaplasma in animals by killing parasites in the bloodstream. However, when administered at its most effective dosage levels, traces of the imidocarb tend to remain in the animal tissue, an undesirable situation if the animal is to be used for human consumption. Therefore, it would be desirable to have a delivery vehicle capable of maintaining the imidocarb in the circulating bloodstream allowing it to perform its therapeutic function without accumulating in the animal tissue.

Imidocarb and its hydrochloric acid salt are hydrophilic compounds, a characteristics which differentiates them from the hydrophobic or hydrophobic/hydrophilic drugs. However, as will be seen from the following Examples 13 and 14, and Figs. 19–21, the microreservoirs are equally effective in beneficially altering the pharmacodynamics of a hydrophilic xenobiotic such as imidocarb.

*EXAMPLE 13*

900 μmoles of egg yolk phosphatidyl choline, 100 μmoles of phosphatidic acid, 100 μmoles of $^3$H-labeled cholesteryl oleate (specific activity $1.6 \times 10^4$ dpm/μmole) and 9.16 mg of $^{14}$C-labeled imidocarb were dissolved in chloroform and the solution was evaporated to dryness under vacuum. The resulting mixed dry residue was then hydrated by the addition of 45 ml of 0.154 M NaCl and 290 mM of trihydroxyethylamine (pH 8.0) to the mixed lipid/drug residue. The liquid was then sonicated for 15 minutes at 51°C under a nitrogen atmosphere.

The sonicated liquid was then chromatographed on a 2.5 × 40 cm Sepharose 4B column.

Individual fractions resulting from the chromatographing were then assayed for phosphatidyl choline and phosphatidic acid by the method of Gomori, and for the $^3$H-cholesteryl oleate and the $^{14}$C-imidocarb by radioactivity. The results of these analyses are plotted in Fig. 19 as a function of fraction number, the analytical results being superimposed as in Fig. 8. The diameter
5 of the vesicular microreservoirs was found to range between about 200 Å and 300 Å.          5
    It can be seen that the elution profiles of the three constituents of the microreservoirs coincide from fractions 5 through 9, indicating that the imidocarb was associated with the microreservoirs.
    The beneficial alteration of the plasma kinetics of imidocarb attained through the use of the
10 delivery vehicles of this invention is illustrated in Example 14 and Figs. 20 and 21.          10

*EXAMPLE 14*
    100 μmoles of phosphatidyl choline and 10 μmoles of phosphatidic acid derived from egg yolk, 10 μmoles of cholesteryl oleate and 2 mg of $^{14}$C-labeled imidocarb were dissolved in
15 chloroform and the solution evaporated to dryness under vacuum. The resulting mixed residue          15
was suspended in 5 ml of 0.154 M NaCl and 10 mM trihydroxymethylamine (pH 8.0) and the resulting suspension was sonicated for 15 minutes at 51°C under a nitrogen atmosphere. The liquid was then chromatographed on a 2.5 × 40 cm Sepharose 4B gel column and the fractions showing a coincidence of the $^{14}$C-labeled imidocarb, phospholipids and cholesteryl oleate were
20 pooled and concentrated by ultrafilatration to a final concentration of 0.6 mg of imidocarb per          20
ml.
    A solution of free imidocarb at a concentration of 0.6 mg/ml in 0.154 M NaCl and 10 mM of trihydroxymethylamine (pH 8.0) was also prepared as a control to be used in free imidocarb administration.
25    The imidocarb-containing microreservoirs and free imidocarb were injected into the tail veins          25
of rats at two dosage levels, ie., 5 mg/kg, and 4.4 mg/kg. Plasma samples were taken at various time points and the amount of imidocarb in these samples was determined by measurements of their radioactivity. The results of these measurements, plotted as imidocarb equivalents as a function of time, are given in Figs. 20 and 21.
30    The beneficial alteration of the plasma kinetics through the use of the circulating microreser-          30
voirs to carry imidocarb in the bloodstream of a living mammalian host is clearly evident from Figs. 20 and 21. For example, at a dosage level of 5 mg/kg, the concentration of the imidocarb in the microreservoirs/imidocarb in the bloodstream was about 140 times greater than the free imidocarb after four hours; and at a dosage level of 4.4 mg/kg about 200 times greater after
35 this same period of time. Moreover, as noted on Fig. 21, the bloodstream still contained a          35
measurable quantity of imidocarb in the microreservoir/imidocarb form after 24 hours as contrasted with virtually none in the free form of imidocarb.
    The ratio of imidocarb to microreservoirs as a function of time is also plotted for each dosage level in Figs. 20 and 21; and these plots clearly show that the drug is released at a rate which
40 can be controlled and predetermined for any given set of conditions. From Figs. 20 and 21 it is          40
also apparent that it is possible, if desired, to reduce the dosage levels of the drug to far below those now considered effective in killing the blood-borne parasites which cause anaplasma in animals.
    One of the major goals in beneficially altering the pharmacodynamics of a xenobiotics is the
45 ability to alter the tissue distribution of the xenobiotic. That this can be achieved for imidocarb          45
using the microreservoirs of this invention is shown in Example 15 and Table 2.

*EXAMPLE 15*
    The animals used in Example 14 were sacrificed at 4 hours after the injection of free
50 imidocarb or the microreservoirs containing imidocarb. Various tissues, such as muscle, spleen,          50
liver and kidney, were taken from the animals. These tissues were combusted in a Searle combustion apparatus and the resulting $^{14}$CO$_2$ was collected and counted for radioactivity. The number of counts per gram of tissue was determined and the results are shown in Table 2.

## TABLE 2
### TISSUE DISTRIBUTION OF FREE IMIDOCARB AND MICRORESERVOIR-BORNE IMIDOCARB

5

| Tissue | Imidocarb | Microreservoir/ Imidocarb | Percent Change |
|--------|-----------|---------------------------|----------------|
| Muscle | 3,985 | 3,511 | − 12 |
| Kidney | 40,194 | 59,984 | + 49 |
| Spleen | 9,498 | 15,339 | + 61 |
| Liver | 27,059 | 29,991 | + 11 |

—dpm/gram of tissue—

The ability to maintain a xenobiotic in the bloodstream and within certain organs is particularly significant for a drug such as imidocarb. Thus, the 12% reduction of imidocarb in muscle tissue attained through the use of microreservoirs is important to the use of this paraciticide in treating animals to be used for human consumption. Moreover, the greater concentration of the drug in the liver and spleen, organs which contain a greater proportion of the anaplasma microorganism, is another significant parameter in exploiting the use of imidocarb.

Among the pharmacodynamics of a xenobiotic which may be beneficially altered by the delivery vehicle of this invention is oral absorption, the altering being achieved by imparting to the xenobiotic the ability to cross the gastro-intestinal (GI) tract and enter into the bloodstream for effective circulation. At present, several solutions are available for administering xenobiotics which do not cross the GI tract, or which cross it to a very limited degree, into the bloodstream. One such solution lies in the chemical modification of the xenobiotic, such as forming the undecanoate ester or estradiol which is essentially insoluble in water and suspending it in oil, forming it into a microcrystalline dispersion or making a solution with an organic solvent such as ethanol.

The incorporation of estradiol undecanoate (used as a fertility control agent) in microreservoirs in accordance with this invention eliminates the need for such liquid media as oils and organic solvents and at the same time achieves a much more satisfactory discharge of this xenobiotic into the bloodstream. The incorporation of estradiol undecanoate in the microreservoirs of this invention is shown in Example 16 and Fig. 22.

### EXAMPLE 16

300 $\mu$moles of egg yolk phosphatidyl choline, 30 $\mu$moles of cholesteryl oleate and 6 $\mu$moles of ^{3}H-labeled estradiol undecanoate (specific activity 4.6 $\mu$Ci/$\mu$mole) were dissolved in benzene and lyophillized. To the resulting dry mixture were then added 5 ml of 0.154 M NaCl and 5 mM trihydroxymethylamine; and the resulting liquid was sonicated for 17 minutes at 48°C under a nitrogen atmosphere. The resulting sonicated mixture was then fractionated by chromatographing it on a Sepharose 4B column to produce the elution profile of Fig. 22. Fractions 12–30 constituted the xenobiotic-containing vesicular microreservoirs used in making an *in vivo* evaluation of the delivery vehicle embodying this invention.

The ability of the microreservoirs embodying this invention to enhance the oral absorption of estradiol undecanoate is further illustrated in Example 17, Fig. 23 and Table 3.

### EXAMPLE 17

[3]H-labeled estradiol undecanoate was encapsulated in microreservoirs composed of egg yolk posphatidyl choline and ^{14}C-labeled cholesteryl oleate. Equivalent amounts of ^{3}H-labeled estradiol undecanoate were dissolved in ethanol. Doses of 1.63 mg/kg were administered orally to two sets of 9 rats. After 1, 4 and 24 hours, three of the rats in each set were killed and the blood collected. The plasma was separated from the red cells by centrifugation and 0.5 ml of each plasma sample was added to 9.5 ml of $CHCl_3$/MeOH (2/1 by volume) containing 1.6 $\mu$moles of unlabeled estradiol and estrone as carriers. The resulting liquid was filtered and sufficient saline was added to the filtrates to make a 2-phase system. The lower phase was taken to dryness and then redissolved in 2.0 ml of methanol. 0.2 ml of this solution was counted for radioactivity. The remaining 1.8 ml was spotted on a TLC plate and developed in benzene/ethyl acetate (3/2 by volume). The plates were exposed briefly to iodine vapor to allow the carrier steroids to become visualized and then iodine vapor was driven off by mild heating. Those spots corresponding to estradiol and estrone were cut out and counted for radioactivity. The equivalents of ^{3}H estradiol undecanoate in the plasma are plotted in Fig. 23. It can be seen that by the first hour the vesicular microreservoirs enhanced the appearance of ^{3}H-labeled estradiol

undecanoate equivalents in the plasma when compared with the ethanol-control solution. After four hours, the amount of estradiol undecanoate equivalents was decreased for both formulations.

5    It is known that estradiol is the active form of the drug, and estrone is inactive. Furthermore, the esters of estradiol are assumed to be inactive until hydrolyzed to estradiol. Therefore, the ratio of estradiol to estrone in plasma should be an index of the ability of the microreservoirs to enhance the active form of the drug.

The results of the TLC analysis of the labeled estradiol derivatives in the plasma are given in Table 3. It can be seen from these data that the ratio of estradiol to estrone is higher by 60% 10  using the microreservoirs than using an ethanol solution.

TABLE 3
RATIO OF ³H ESTRADIOL TO ³H ESTRONE IN PLASMA ONE HOUR AFTER ORAL ADMINIS-
TRATION OF ESTRADIOL UNDECANOATE

| Formulation | Estradiol/Estrone* |
|---|---|
| In ethanol solution | 1.14 |
| In microreservoirs | 1.84 |

*Ratio is based on average of three rats.

The microreservoir delivery system embodying this invention also offers the possibility of orally administering xenobiotics heretofore incapable of this mode of administration. By 25  transporting such drugs across the GI tract it is possible to deliver them to the bloodstream indirectly, thus eliminating their metabolism in the GI tract and the need for injections and the trouble and complications attendant on this form of administration.

The microreservoirs may be used in either their vesicular form or nonvesicular form or in a combination of these forms. This is illustrated by Example 18 and Figs. 24 and 25.

EXAMPLE 18
25 μmoles of egg phosphatidyl choline, 75 μmoles of ¹⁴C-labeled cholesteryl oleate, and 2 μmoles of ³H-labeled estradiol undecanoate were dissolved in chloroform and taken to dryness. The lipid mixture was dried overnight under vacuum and then hydrated with 4.4 ml of 0.154 M 35  NaCl and 5 mM trihydroxymethylamine (pH 7.2). The resulting liquid was sonicated at 40°C for 20 minutes under a nitrogen atmosphere and passed down a Sepharose 4B Column. The resulting elution profile is shown in Fig. 24. It can be observed that the estradiol undecanoate has a higher affinity for the vesicular form of the microreservoirs than for the nonvesicular form, based on the ratio of estradiol undecanoate to cholesteryl oleate in the two forms of the 40  microreservoirs. The material in the void volume of the column consisted of the nonvesicular form, whereas the material in the internal volume consisted of the vesicular form.

Samples of both the nonvesicular and vesicular forms containing estradiol undecanoate and approximately one μmole of total lipid were incubated with 9 μmoles of an unsonicated egg phosphatidyl choline dispersion. At various time points the samples were centrifuged at 45  15,000g for 2 minutes and the supernatant containing the microreservoirs was counted for radioactivity. Since the cholesteryl oleate is a nonexchangeable species in this system, the ratio of ³H-labeled estradiol undecanoate to ¹⁴C-labeled cholesteryl oleate is an indication of the rate of efflux of estradiol undecanoate from the nonvesicular or vesicular microreservoirs to the phosphatidyl choline dispersion. The results are shown in Fig. 25. It will be seen that the 50  estradiol undecanoate remained associated with both forms of the microreservoirs, indicating that both are satisfactory for carrying and releasing xenobiotics.

The xenobiotic-containing microreservoirs embodying this invention may be formulated into a variety of dosage forms. Thus, for example, they may be dispersed in a physiologically compatible liquid, they may be used dry to form tablets, or they may be contained in capsules 55  formed of a suitable biocompatible material.

From the detailed description and examples given, it will be seen that the delivery vehicle of this invention is capable of beneficially altering the pharmacodynamics of xenobiotics having a wide range of chemical and physical characteristics as well as a wide range of biological uses and properties.

60    It will thus be seen that the objects set forth above, among those made apparent from the preceding description, are efficiently attained and, since certain changes may be made in carrying out the above method and in the composition and article set forth without departing from the scope of the invention, it is intended that all matter contained in the above description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting 65  sense.

CLAIMS

1.  A delivery vehicle biocompatible with a mammalian host to deliver and release within said host a xenobiotic the pharmacodynamics of which are beneficially altered through the use of said vehicle, characterized in that said delivery vehicle is in the form of microreservoirs containing said xenobiotic, said microreservoirs comprising a phospholipid constituent and a phospholipid-immiscible lipid constituent stable in and essentially immiscible with a physiologically-compatible liquid.

2.  A delivery vehicle in accordance with claim 1 wherein said microreservoirs are in vesicular form having diameters ranging between about 190 Å and about 300 Å.

3.  A delivery vehicle in accordance with claim 1 wherein said microreservoirs are in nonvesicular form having diameters ranging between about 250 Å and about 1000 Å.

4.  A delivery vehicle in accordance with claim 1 wherein said microreservoirs are in both nonvesicular and vesicular forms.

5.  A delivery vehicle in accordance with claim 1 wherein said microreservoirs comprise between about 50 mole % and about 97 mole % of said phospholipid constituent.

6.  A delivery vehicle in accordance with claim 1 wherein said microreservoirs include a xenobiotic binding modifier.

7.  A delivery vehicle in accordance with claim 6 wherein said xenobiotic binding modifier increases the pickup of said xenobiotic by said microreservoirs.

8.  A delivery vehicle in accordance with claim 7 wherein said xenobiotic binding modifier is phosphatidic acid forming a minor portion of said phospholipid constituent.

9.  A delivery vehicle in accordance with claim 6 wherein said xenobiotic binding modifier decreases the pickup of said xenobiotic by said microreservoirs.

10.  A delivery vehicle in accordance with claim 9 wherein said xenobiotic binding modifier is cholesterol.

11.  A delivery vehicle in accordance with claim 1 wherein said microreservoirs include a xenobiotic release-rate control agent.

12.  A delivery vehicle in accordance with claim 11 wherein said xenobiotic release-rate control agent is a lipid miscible with said phospholipid constituent of said microreservoirs.

13.  A delivery vehicle in accordance with claim 12 wherein said lipid is cholesterol.

14.  A delivery vehicle in accordance with claim 1 wherein said phospholipid constituent comprises phosphatidyl choline.

15.  A delivery vehicle in accordance with claim 1 wherein said phospholipid constituent comprises a mixture of phosphatidyl choline and phsophatidic acid, said phosphatidic acid being present in said mixture in an amount equivalent to at least about 5 mole %.

16.  A delivery vehicle in accordance with claim 1 wherein said phopsholipid-immiscible lipid constituent comprises a cholesterol ester of a fatty acid having between 10 and 18 carbon atoms.

17.  A delivery vehicle in accordance with claim 16 wherein said cholesterol ester is cholesteryl oleate.

18.  A delivery vehicle in accordance with claim 1 wherein said phospholipid-immiscible lipid constituent comprises a triglyceride.

19.  A delivery vehicle in accordance with claim 18 wherein said triglyceride is glycerol trioleate.

20.  A delivery vehicle in accordance with claim 1 wherein said xenobiotic is a drug.

21.  A delivery vehicle in accordance with claim 20 wherein said drug is a chemotherapeutic drug.

22.  A delivery vehicle in accordance with claim 21 wherein said drug is a cancer chemotherapeutic drug.

23.  A delivery vehicle in accordance with claim 22 wherein said cancer chemotherapeutic drug is an anthracycline.

24.  A delivery vehicle in accordance with claim 23 wherein said cancer chemotherapeutic drug is adriamycin.

25.  A delivery vehicle in accordance with claim 23 wherein said cancer chemotherapeutic drug is AD 32.

26.  A delivery vehicle in accordance with claim 20 wherein said drug is a parasiticide.

27.  A delivery vehicle in accordance with claim 26 wherein said parasiticide is imidocarb.

28.  A delivery vehicle in accordance with claim 20 wherein said drug is a fertility control agent.

29.  A delivery vehicle in accordance with claim 28 wherein said fertility control agent is estradiol undecanoate.

30.  A delivery vehicle in accordance with claim 1 wherein said xenobiotic is a drug, the plasma kinetics of which are beneficially altered.

31.  A delivery vehicle in accordance with claim 1 wherein said xenobiotic is a drug, the

chemotherapeutic effectiveness of which is beneficially altered.

32.  A delivery vehicle in accordance with claim 1 wherein said xenobiotic is a drug, the toxicity of which is beneficially altered.

33.  A delivery vehicle in accordance with claim 1 wherein said xenobiotic is a drug, the oral
5 absorption, and hence its ability to pass the gastrointestinal tract, is beneficially altered.                                 5

34.  A method of forming a delivery vehicle as claimed in any preceding claim, comprising the steps of forming microreservoirs of a composition comprising a phospholipid constituent and a phsopholipid-immiscible lipid constituent which is stable in and essentially immiscible with a physiologically-compatible liquid; and incorporating said xenobiotic to be delivered within said
10 microreservoirs.                                                                                                            10

35.  A method in accordance with claim 34 wherein said step of forming said microreservoirs comprises

(a) forming with a solvent a solution of said phospholipid constituent and of said phospholipid-immiscible lipid constituent;
15 (b) removing said solvent to produce a dry residue mixture of said phospholipid constituent          15
and said phospholipid-immiscible lipid constituent;

(c) hydrating said dry residue mixture with a physiologically-compatible liquid to form a suspension;

(d) sonicating said suspension under a nonoxidizing atmosphere at a temperature at least
20 equivalent to the melting point of said phospholipid-immiscible lipid constituent to form said        20
microreservoirs; and

(e) separating out said microreservoirs thus formed.

36.  A method in accordance with claim 35 wherein said step of incorporating said xenobiotic within said microreservoirs comprises adding said xenobiotic to said solution in step
25 (a).                                                                                                                        25

37.  A method in accordance with claim 35 wherein said step of incorporating said xenobiotic within said microreservoirs comprises adding said xenobiotic to said suspension of step (c) prior to said sonicating.

38.  A method in accordance with claim 35 wherein said step of separating out said
30 microreservoirs thus formed comprises centrifuging the sonicated suspension and chromato-          30
graphing the clear phase resulting from said centrifuging to provide a series of chromatographed fractions.

39.  A method in accordance with claim 38 further including the step of separating those of said chromatographed fractions containing said microreservoirs in vesicular form having diame-
35 ters ranging between about 190 Å and 300 Å from those fractions containing said microreser-          35
voirs in nonvesicular form having diameter ranging between about 250 Å and about 1000 Å.

40.  A method in accordance with claim 35 including the step of adding a xenobiotic binding modifier to said solution.

41.  A method in accordance with claim 35 including the step of adding a xenobiotic release-
40 rate control agent to said solution.                                                                                         40

42.  A method in accordance with claim 34 wherein said step of forming said microreservoirs comprises

(1) forming in a water-miscible organic solvent a solution of said phospholipid constituent and said phospholipid-immiscible lipid constituent;
45 (2) injecting said solution into said physiologically-compatible liquid under conditions to form          45
said microreservoirs.

43.  A method in accordance with claim 42 wherein said step of incorporating said xenobiotic within said microreservoirs comprises adding said xenobiotic to said solution.

44.  A method in accordance with claim 42 wherein said step of incorporating said
50 xenobiotic within said microreservoirs comprises adding said xenobiotic to said physiologically-      50
compatible liquid.

45.  A method in accordance with claim 34 including the step of suspending said microreservoirs containing said xenobiotic in said physiologically-compatible liquid thereby providing said xenobiotic in liquid dosage form.
55 46.  A method in accordance with claim 34 including the step of encapsulating said                          55
microreservoirs containing said xenobiotic in a capsule formed of a physiologically-acceptable material.

47.  A method of delivering and releasing a xenobiotic within a mammalian host, comprising the step of introducing into a mammalian host a pharmaceutically effective amount of a
60 xenobiotic contained within microreservoirs formed of a phospholipid constituent and a              60
phospholipid-immiscible lipid constituent stable in and essentially immiscible with a physiologi-cally-compatible liquid.

48.  A method of predetermining and controlling the pharmacodynamics under which a xenobiotic is delivered within a mammalian host, comprising the step of releasing said
65 xenobiotic within said host from circulating microreservoirs formed of a phospholipid constituent     65

and a phospholipid-immiscible lipid constituent stable in and essentially immiscible with a physiologically-compatible liquid.

49.   Delivery vehicles substantially as hereinbefore described.

50.   The methods of forming delivery vehicles substantially as hereinbefore described.

5    51.   Delivery vehicles made by the method claimed in any of claims 34 to 48, or 50.                     5